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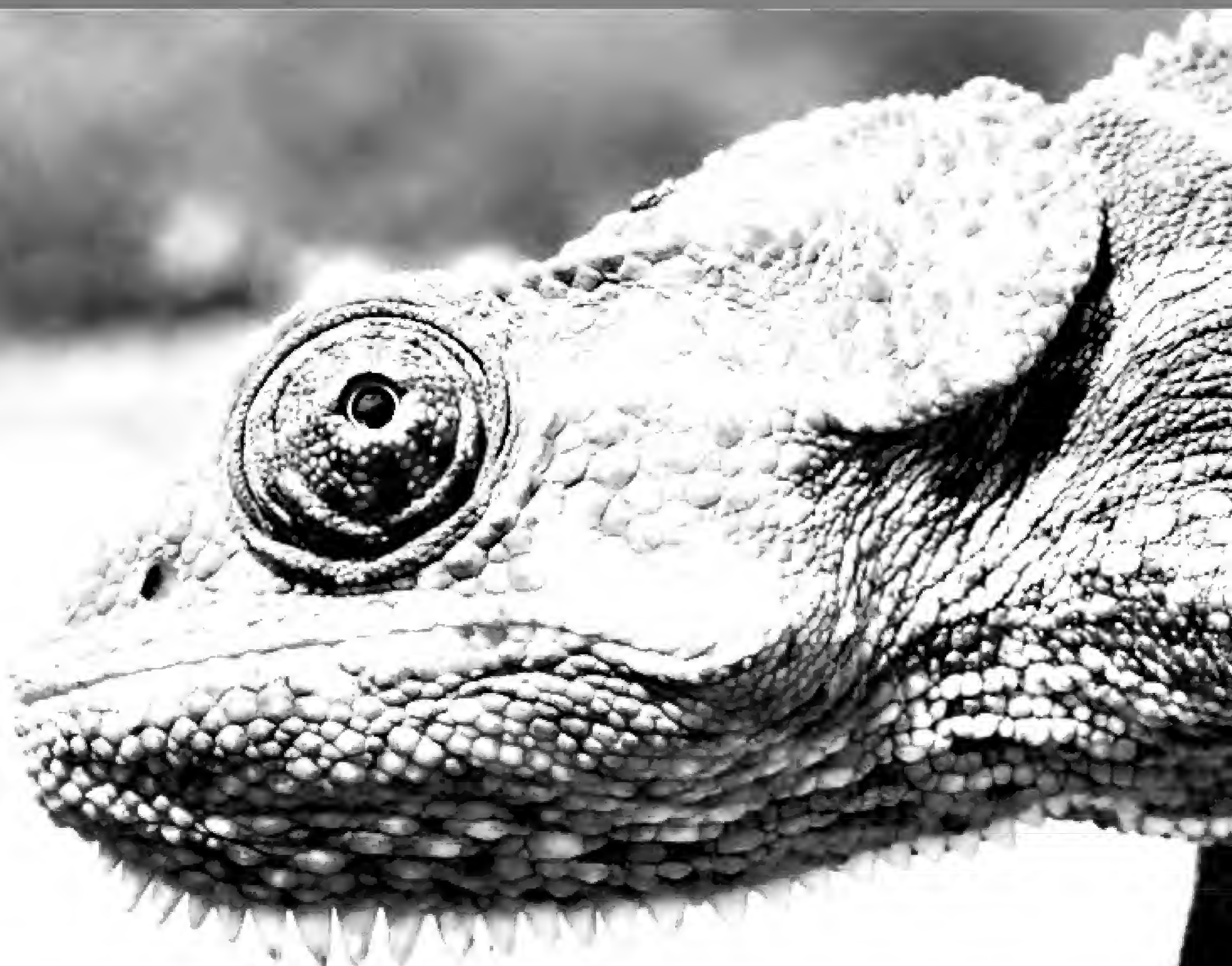
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*Chamaeleo monachus* Gray, 1865 - Socotra Island (Yemen)



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Cover: An adult male of *Chamaeleo monachus*, Socotra Island, Wadi Ayheft, II.2009. 1) idem. 2) *Pristurus sokotranus*, Socotra Island, Wadi Da'Arho, II.2009. 3) *P. samhaensis*, Darsa Island, II.2009. (photos by Pietro Lo Cascio and Flavia Grita)

**REPTILES OF SOCOTRA.** *Chamaeleo monachus* was described by the herpetologist John E. Gray in 1865, who indicated “Madagascar” as type-locality for the new species. However, the specimen studied by Gray came from Socotra, where chameleons were perhaps collected as pets by Arab sailors and successively sold to British travellers with erroneous information about their provenience, but only after the first scientific expedition carried out on the island by the botanist Isaac Balfour in 1880 it was possible to determine its true origin. *C. monachus* now is appropriately known as one of the several endemic reptile species of the Socotra Archipelago (Yemen), where it is the only representative of the family Chamaeleonidae and where it is exclusively distributed on the main island. The archipelago is located about 380 kilometers south-east off the Yemen coast and 100 km east from Cape Guardafui (Somalia), and includes four islands, whose size ranges from 3,625 (Socotra) to 12 km<sup>2</sup> (Darsa). Socotra’s levels of endemism confer global significance, both in plants and animals; the main island is a fragment of Gondwana, firstly isolated in the Indian Ocean during Eocene-Oligocene (34-41 million years ago), and palaeogeographic data indicate that all the islands have been definitively isolated from Africa about six million years ago. Reptiles is undoubtedly one of the most important and significant groups among the vertebrate faunas of these islands in terms of biological diversity. According to the recently updated checklist given by Razzetti *et al.* (2011, in Zootaxa 2826: 1-44), the Socotra Archipelago harbours 30 species belonging to 12 different genera, some of which are strictly endemic of the islands: the gekkonid *Haemodracon* Bauer *et al.*, 1997, and two snake monotypic genera, the colubrid *Hemerophis* Schätti & Utiger, 2001, and the lamprophiid *Dityophis* Günther, 1881. Except for the bizarre story of the homeland of the Socotran chameleon, the first knowledge on the herpetofauna of the archipelago is mainly due to the zoological expedition led by the British naturalists Henry O. Forbes and William R. Ogilvie-Grant in the late 19th century, but investigations on taxonomy and distribution of several species are still in progress, as evidenced by the recent description of the gekkonid *Hemidactylus inintellectus* Sindaco *et al.*, 2009, as well as by the fact that seven other species have been described during the last three decades. The endemism rate among reptiles is very high and 90% of occurring species are exclusive of one or more islands; moreover, some of which are also strictly confined on very small areas: a significant example is given by *Hemidactylus dracaenaculus* Rösler & Wranik, 1999, so far known only from few localities of the Diksam Plateau at Socotra where it inhabits barks and trunks of the renowned dragon blood trees, the relictual endemic *Dracaena cinnabari*. Most part of the occurring reptiles (18) belong to the family Gekkonidae and some genera, such as the diurnal Semaphore geckos *Pristurus* Rüppell, 1835 or the nocturnal *Hemidactylus* Oken, 1817, are interested by remarkable processes of adaptive radiation: both include 7 endemic species (the latter, also, comprises 3 species introduced on the islands). In particular, Socotra and its satellite islands harbour one third of the 20 recognised species of *Pristurus*, a genus distributed in Arabia and north-eastern Africa with an isolate in Mauritania. These geckos are mainly heliothermic ground- or rock-climbers, but a small number of taxa is known as tree dwelling; among the Socotran representatives, *P. obsti* Rösler & Wranik, 1999, originally recorded for the mangroves of Shu’ab Gulf, and the closely related *P. guichardi* Arnold, 1986, known for the mountains of Hajhir Massif, are purely arboreal, while the most common and widespread *P. sokotranus* Parker, 1938, as well as *P. insignis* Blanford, 1881 and *P. insignoides* Arnold, 1986, are generally associated to rocks and cliffs. *P. abdelkuri* Arnold, 1986 is endemic of the westernmost island Abd al-Kuri, but some introduced populations of this species have been recently recorded at Socotra. Finally, *P. samhaensis* Rösler & Wranik, 1999 replaces *P. sokotranus* in the small islands of Samha and Darsa, also called “The Brothers”.

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## Exotic molluscs (Mollusca, Gastropoda et Bivalvia) in Santa Catarina State, Southern Brazil region: check list and regional spatial distribution

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**ABSTRACT** A total of twenty-one exotic mollusc taxa were assessed for Santa Catarina State (SC), fifteen Gastropoda and six Bivalvia (twelve terrestrial, five limnic/freshwater - three gastropods and two bivalves, and four marine bivalves). Of these, fourteen are confirmed as invasive species (nine terrestrial, three limnic/freshwater, and two marine).

**KEY WORDS** Biodiversity, Continental mollusc fauna, Exotic and invasive species, Santa Catarina State, Southern Brazil region

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### INTRODUCTION

To date, the presence of a total of twenty-one (21) mollusc species, under the designation of “exotic introduced species” (48% of the total acknowledged in Brazil), was confirmed for the territory of Santa Catarina State (SC), a small central state within the South Brazil region – of these species, fifteen were Gastropoda and six Bivalvia (twelve terrestrial, five limnic/freshwater – three gastropods and two bivalves – four marine bivalves). The list also includes the slug *Pallifera* sp., a species still within the taxonomic status confirmation process, with descriptions of the species to be found in Agudo & Bleicker (2006), Agudo-Padrón (2008a) and Agudo-Padrón & Lenhard (2010). Of these species, fourteen are identified as invading forms in Santa Catarina State (ten Gastropoda – nine terrestrial and one freshwater – and four Bivalves – two freshwater and two marine). In the present work, the current regional knowledge situation of these molluscs is briefly revised, including basic maps covering the distribution of such species in the state.

### ANALYSIS OF THE CONTEMPLATED SITUATION

The current survey started in November 2009 and included the organization of official seminars (Official State Program for Listing and Control of

Invasive Exotic Species), organized and driven by the Official Santa Catarina State Environment Foundation (Fundação do Meio Ambiente – FATMA) jointly with the Hórus Institute of Development and Environmental Conservation (Instituto Hórus de Desenvolvimento e Conservação Ambiental). The main goal of such seminars was the formulation of a “Official State List of Species” (Agudo-Padrón 2011a, b).

Of the two participant researchers in the enacted Mollusc Group, only one worked specifically with continental species. It is worth highlighting that the Asian golden mussel, *Limnoperna fortunei* (Dunker, 1857), a highly invasive species which is still localized within Santa Catarina State (Agudo-Padrón 2007, 2008b; Agudo-Padrón & Lenhard 2010), received particular attention within such seminars. On another note, the cultivated mussel *Perna perna* (Linnaeus, 1758) was removed from the list of invasive species for the State since, after an extensive analysis and technical discussion, it was concluded that the species is actually being considered a native one in the State and in the whole of Brazil (Magalhães et al., 2007; Schaefer et al., 2009).

The following is a list of introduced and invading molluscs in Santa Catarina State (SC) along with inter-relationships between such species, based mainly on the taxonomic contributions of Simone (2006) and Thomé et al. (2006, 2007) (Figs 1-17).



*Rumina decollata*

Fig.1



*Vertigo ovata*

Fig.2



*Pallifera sp.*

Fig.3



*Lehmannia valentiana*

Fig.4



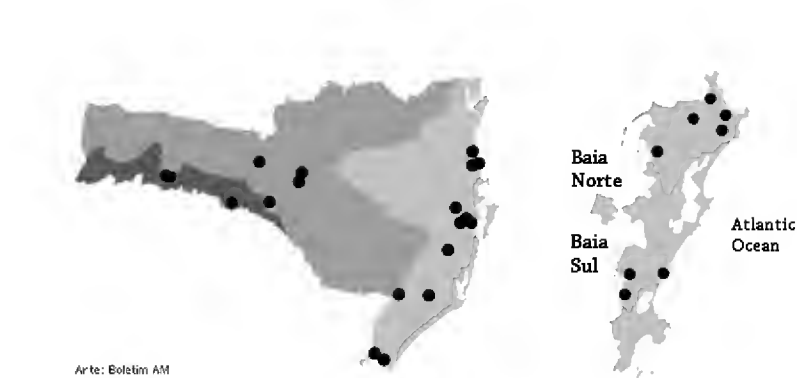
*Limacus flavus*

Fig.5



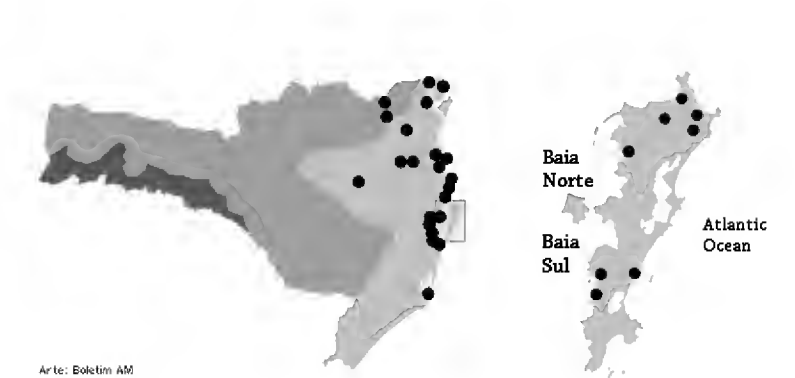
*Limax maximus*

Fig.6



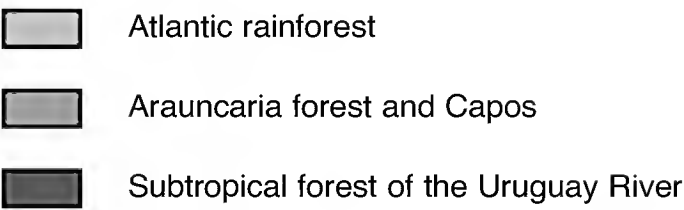
*Deroceras laeve*

Fig.7

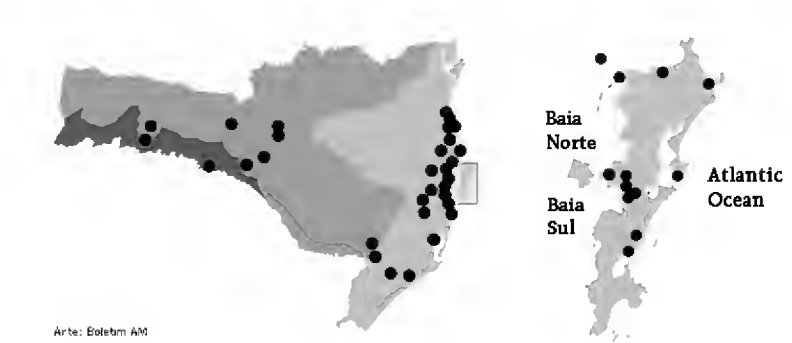


*Achatina fulica*

Fig.8



Figures 1-8. Regional spatial distribution of exotic molluscs in Santa Catarina (1).



*Bradybaena similaris*

Fig.9



*Helix (Cornu) aspersa*

Fig.10



*Paralaoma servilis*

Fig.11



*Zonitoides arboreus*

Fig.12



*Pomacea paludosa*

Fig.13



*Melanoides tuberculatus*

Fig.14



*Aplexa rivalis*

Fig.15



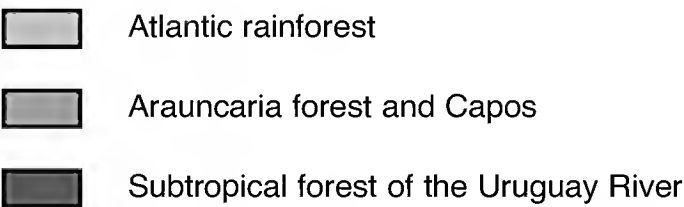
*Corbicula fluminea*

Fig.16



*Corbicula largillierti*

Fig.17



Figures 9-17. Regional spatial distribution of exotic molluscs in Santa Catarina (2).



## RESULTS

### TERRESTRIAL TAXA

Twelve recognized species (26% of the total confirmed in Brazil). Of these, nine are specific invading forms.

#### Class GASTROPODA - Pulmonata

Family SUBULINIDAE Thiele, 1931

Genus *Rumina* Risso, 1826

*Rumina decollata* (Linnaeus, 1758)

Family VERTIGINIDAE Fitzinger, 1833

Genus *Vertigo* Müller, 1774

*Vertigo ovata* Say, 1822

Family PHILOMYCIDAE Keferstein, 1866

Genus *Pallifera* Morse, 1864

*Pallifera* sp. (Fig. 18)

INVADER

Family LIMACIDAE Rafinesque, 1815

Genus *Limacus* Lehmann, 1864

*Limacus flavus* (Linnaeus, 1758) (Fig. 19)

INVADER

Genus *Limax* Linnaeus, 1758

*Limax maximus* Linnaeus, 1758 (Fig. 20)

INVADER

Genus *Lehmannia* Heynemann, 1863

*Lehmannia valentiana* Férussac, 1822

INVADER

Family AGRIOLIMACIDAE Wagner, 1935

Genus *Deroceras* Rafinesque, 1820

*Deroceras laeve* (Müller, 1774)

INVADER

Family ACHATINIDAE Swainson, 1840

Genus *Achatina* Lamarck, 1799

*Achatina (Lissachatina) fulica* (Bowdich, 1822)

INVADER

Family BRADYBAENIDAE Pilsbry, 1934

Genus *Bradybaena* Beck, 1837

*Bradybaena similaris* (Férussac, 1821) (Fig. 21)

INVADER

Family HELICIDAE Rafinesque, 1815

Genus *Helix* Linnaeus, 1758

*Helix (Cornu) aspersus* (Müller, 1774) (Fig. 22)

INVADER

Family PUNCTIDAE Morse, 1864

Genus *Paralaoma* Iredale, 1913

*Paralaoma servilis* (Shuttleworth, 1852)

Family GASTRODONTIDAE Tryon, 1866

Genus *Zonitoides* Lehmann, 1862

*Zonitoides arboreus* (Say, 1817)

INVADER

### FRESHWATER/ LIMNIC TAXA

Five recognized species (12% of the total confirmed in Brazil). Of this, three are specific invading forms.

#### Class GASTROPODA

Caenogastropoda

Family AMPULLARIIDAE Gray, 1824

Genus *Pomacea* Perry, 1811

*Pomacea paludosa* (Say, 1829)

Family THIARIDAE Troschel, 1857

Genus *Melanoides* Olivier, 1804

*Melanoides tuberculatus* (Müller, 1774)

INVADER

Pulmonata

Family PHYSIDAE Fitzinger, 1833

Genus *Aplexa* Fleming, 1820

*Aplexa rivalis* (Maton & Rackett, 1807)

#### Class BIVALVIA - Veneroida

Family CORBICULIDAE Gray, 1847

Genus *Corbicula* Megerle von Mühlfeld, 1811

*Corbicula fluminea* (Müller, 1774) (Fig. 23)

INVADER

*Corbicula largillierti* (Philippi, 1844)

INVADER

### MARINE TAXA

Four recognized species (9% of the total confirmed in Brazil). Of these, two are specific invading forms.

#### Class BIVALVIA

Ostreoida

Family OSTREIDAE Rafinesque, 1815

Genus *Crassostrea* Sacco, 1897

*Crassostrea gigas* (Thunberg, 1795)

*Crassostrea virginica* (Gmelin, 1791)



Fig.18



Fig.19



Fig.20



Fig.21



Fig.22



Fig.23

Figure 18. Invasive exotic slugs *Pallifera* sp.

Figure 19. *Limacus flavus*.

Figure 20. *Limax maximus* (photo P. Lenhard).

Figure 21. *Bradybaena similaris* (photo P. Lenhard).

Figure 22. *Cornu aspersum* (photo P. Lenhard).

Figure 23. *Corbicula fluminea*.

## Pterioida

Family ISOGNOMONIDAE Woodring, 1925

Genus *Isognomon* Lightfoot, 1786

*Isognomon bicolor* (C. B. Adams, 1845)

INVADER

## Mytiloida

Family MYTILIDAE Rafinesque, 1815

Genus *Lithophaga* Roding, 1798

Subgenus *Myoforceps* P. Fischer, 1886

*Lithophaga (Myoforceps) aristatus* (Dillwyn, 1817)

INVADER

## DISCUSSION AND CONCLUSIONS

The official lists of alien and invasive mollusc species for Santa Catarina State compiled by regional environment institutions (CONSEMA 2010) overlook or give scant importance to the species listed in this manuscript, listing only a total number of six related species, five of them being recognized as “invasive forms” in the State (two terrestrial = *Achatina fulica*, *Helix aspersa*; three freshwater/limnic = *Melanoides tuberculatus*, *Corbicula fluminea*, *Corbicula largillierii*; and one marine = *Crassostrea gigas*).

It is hoped that soon this situation is properly reviewed, corrected and updated.

## ACKNOWLEDGEMENTS

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## **Threatened freshwater and terrestrial molluscs (Mollusca, Gastropoda et Bivalvia) of Santa Catarina State, Southern Brazil: check list and evaluation of regional threats**

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**ABSTRACT** A total of nineteen continental native mollusc species are confirmed for the Santa Catarina State (SC) (organized in ten Genera and seven Families), one aquatic Prosobranchia/Caenogastropoda (Ampullariidae), six Pulmonata terrestrial gastropods (one Ellobiidae, three Megalobulimidae and two micro-snails – Charopidae and Streptaxidae) and twelve freshwater mussels (eight Mycetopodidae and four Hyriidae). These species are designated by the International Union for Conservation of the Nature – IUCN as follows: seven as "Vulnerable", six "In Danger" and six “Without Category Established”. The general regional threats that these species are subjected to are briefly analyzed.

**KEY WORDS** Biodiversity, Continental mollusc fauna, Threatened species, Santa Catarina State, Southern Brazil region

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### **INTRODUCTION**

In spite of prodigious scientific and technological progress in recent years, in throughout Brazil and other Neotropical countries, significant difficulties in evaluating the threats impinging on continental-terrestrial and freshwater-molluscs species are constantly being faced by the scientific community, especially in the geo-political territory of Santa Catarina State (SC), the smallest space portion of the Southern Brazil mosaic (Agudo & Bleicker, 2006a; Agudo-Padrón, 2006; Agudo, 2007a; Agudo-Padrón, 2007a, 2008a, 2009a, b; Agudo-Padrón & Bleicker, 2009). This state of affairs is mainly due to the lack of solid population data and to the small amount of resident limnologists in this State.

Nowadays, the Santa Catarina State authorities govern in this territory nine State Ecological Units of Conservation - six belonging to the category “Park”, where access to the public is permitted in most areas, and three belonging to the category “Reserve”, where

access is quite restricted and permitted only to researchers; this besides four “National Ecological Parks” within the jurisdiction of the same State.

However, do such protected areas truly result in effective conservation of our known continental malacological species and of species which to date have yet to be described?

As previously noticed by local limnologists (Moraes, 2006), all of the Brazilian native mollusc species are in imminent threat of extinction, besides forms that are still awaiting discovery. Considering the rapid rate of anthropogenic environmental degradation, it can be hypothesized that a number of such species have gone extinct before they were at least recorded and described scientifically (Simone, 2006).

Besides the environmental degradation (through deforestation for agricultural ends and/or mining exploration, pollution of the river basins with discharges of organic and inorganic pollutants, indiscriminate application of agricultural poisons and chemical fertilizers,

proliferation of the construction of hydroelectric mills, invasions of natural spaces by town planning enterprises), the Brazilian terrestrial mollusc species face stiff competition by invading forms, that are also responsible for serious sanitary and agronomic problems, among others (Agudo, 2007b; Agudo & Bleicker 2006b; Agudo-Padrón 2006, 2007a, b, 2008b, c, d; Agudo-Padrón & Lenhard, 2010). Brought to Brazil willfully for a variety of purposes, or even accidentally, those exotic species are alien to the local ecosystem and for this reason they don't possess natural predators, resulting in an uncontrolled growth of the population, that, consequently, smothers and even obliterates native species through the usurpation of their niches (Simone, 2002).

That scenario is worsened by the absence of any awareness on the conservation status of these animals, which are generally not considered charismatic enough so as to warrant the declaration of protected natural areas - the molluscs have a very smaller appeal to the population than megafaunal species, in spite of being fundamental for the ecological balance of ecosystems (Moraes, 2006) (Figs 1-11).

During the course of this study, we also had the opportunity to document personally the change in fortunes of some iconic terrestrial mollusc species - for instance the native giant snail *Megalobulimus gummatus* (Hidalgo, 1870) (Fig. 2), found mainly in the valley of the Uruguay river basin. Abundant previously at the same location, today it results difficult to track down in the local environment, as a result of the increase in regional agricultural activities (application of pesticides, mainly); meanwhile invading exotic species, such as the slug *Pallifera* sp. (Fig. 1), proliferate and colonise new areas.

In other cases (very rare), native species resist and adapt to the anthropological conditions imposed in their natural environment when this is invaded becoming themselves, in turn, agricultural pests in small vegetable cultures. An example of this situation is presented by the case of the giant native snail *Megalobulimus oblongus* (Müller, 1774) (Fig. 3), in sandbanks of the "Enseada do Brito", Palhoça Municipal District of the Great Florianópolis, a traditional village of artisanal fishermen located in the proximities of the "Serra do Tabuleiro Ecological State Park" (Agudo-Padrón & Bleicker, 2009).

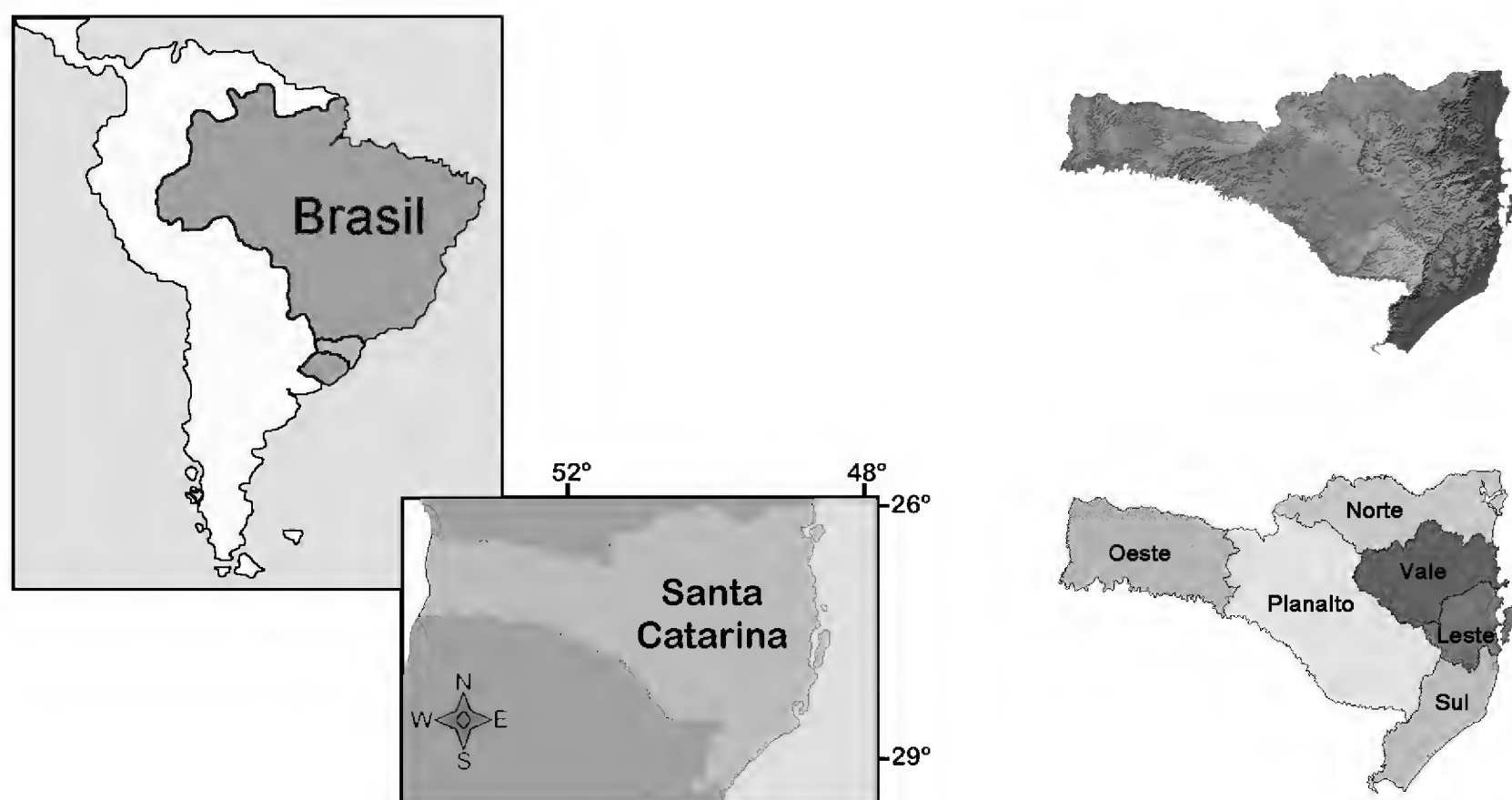


Table 1. Santa Catarina State, SC, central portion of the Southern Brazilian country (on the left), and regional geopolitical division showing physical, socioeconomic and environmental (phytogeographical) characteristics (on the right). Santa Catarina lies between latitudes 25° and 30° S and longitudes 48° and 54° W, extends 377 km from North to South and 547 km from East to West at its most distant points, and has an area of 95,985 km<sup>2</sup>, which includes 502 km<sup>2</sup> of rivers and lakes. The state constitutes only 1.13% of the total area of Brazil and is divided geographically into three large parts: the Atlantic Coastal Plains, with several rivers that discharge into the Atlantic Ocean, and two independent great river basin systems that irrigate the land in the central and western highlands, the Iguazu and the Uruguay.



Fig.1



Fig.2

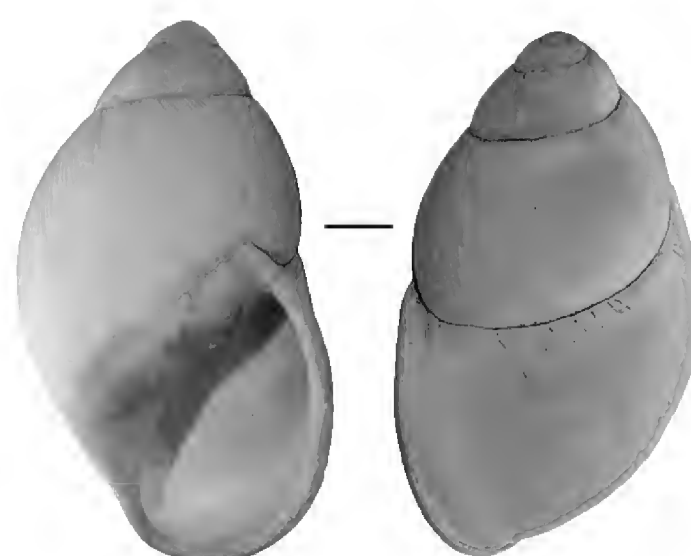


Fig.3

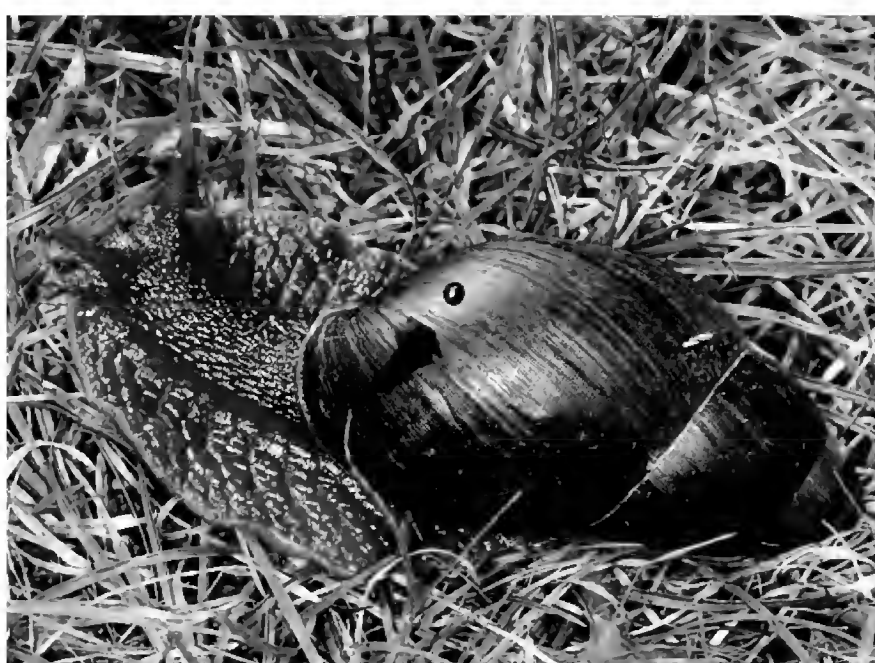


Fig.4



Fig.5

- Figure 1. Invasive exotic Asian slugs *Pallifera* sp.  
 Figure 2. Native giant snail *Megalobulimus gummatus*, 108 mm.  
 Figure 3. Native giant snail *Megalobulimus oblongus*, 70 mm (photos: P. Lenhard).  
 Figure 4. Native giant snail *Megalobulimus grandis*, 130 mm (photo: G. Woehl Jr.).  
 Figure 5. Native snails *Megalobulimus proclivis*, 86 mm.

Curious situation comes with the involvement of the giant freshwater native bivalve *Anodontites trapesialis* (Lamarck, 1819) in the Northern region of the State (Joinville Municipal District) and other Brazilian localities out of the State, whose parasitic larvae type “Lasidium” are undesirable and harmful pests in enterprises fish farmers (Agudo, 2005, 2008).

According to Mansur et al. (2003) and Mansur (2008), it just is not enough to place the native species in lists of those threatened by extinction: it is necessary to know our native fauna from the taxonomic, morphologic and ecological point of view so as to be able to propose handling and management strategies.

As previously noted, an inefficient administration and man’s growing need for water are bringing freshwater ecosystems to the collapse, making freshwater species the most threatened of the planet.

The molluscs that live in rivers and lakes are the most threatened of the Earth, due to the collapse of aquatic ecosystems mediated by the construction of dams and through the incessant siphoning off of water for agriculture and other purposes. The rates of extinction of species in freshwater environments are from four to six times higher than in marine or terrestrial habitats. Endemic species, such as the small aquatic snail *Potamolithus catharinae* Pilsbry, 1911, representative of the Family Hydrobiidae (Silva & Veitenheimer-Mendes, 2004), and the tiny freshwater limpets *Burnupia ingae* Lanzer, 1991 and *Ferrissia gentilis* Lanzer, 1991 (Family Ancyliidae), are particularly vulnerable to human alterations of their environment (Agudo-Padrón, 2011a, b).

The freshwater bivalve molluscs are particularly sensitive to trampling, to organic and chemical pollution, and other forms of degradation of the environment. They present relatively slow growth rates and they don’t usually occupy disturbed environments. Endemic species exist for each basin and many of these are very restricted spatially and present high rates of extinction due to the countless environmental alterations provoked recently by human settlement.

In the present work, the current regional knowledge situation of these mollusc species is revised, including IUCN general status and other information, to promote their effective conservation.

## RESULTS

### CURRENT SITUATION

Class GASTROPODA

Subclass PROSOBRANCHIA/CAENO-GASTROPODA

Family AMPULLARIIDAE

*Pomacea sordida* Swainson, 1823

Category IUCN: without category established

Included in the “Lista das Espécies da Fauna Ameaçadas de Extinção no Estado do Rio de Janeiro - RJ” (1997), regional category “in danger”.

Subclass PULMONATA

Family ELLOBIIDAE

*Melampus coffeus* (Linnaeus, 1758)

Category IUCN: without category established

Reported in the “Lista das Espécies da Fauna Ameaçadas de Extinção no Estado do Rio de Janeiro - RJ” (1997), regional category “Vulnerable”. Species considered a “marine form with wide ecological occurrence”.

Family MEGALOBULIMIDAE

*Megalobulimus grandis* (Martens, 1885) (Fig. 4)

Category IUCN: in danger

*Megalobulimus proclivis* (Martens, 1888) (Fig. 5)

Category IUCN: in danger

*Megalobulimus oblongus* (Müller, 1774)

Category IUCN: without category established

Recently included in the “Lista de Espécies da Flora e da Fauna Ameaçadas no Estado do Pará - PA” (2007), regional category “in danger”.

Family CHAROPIDAE

*Rotadiscus schuppi* (Suter, 1900)

Category IUCN: in danger

Family STREPTAXIDAE





Fig.6



Fig.7

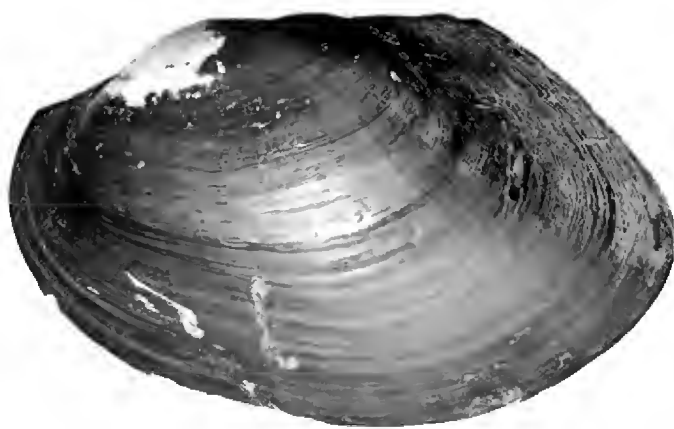


Fig.8

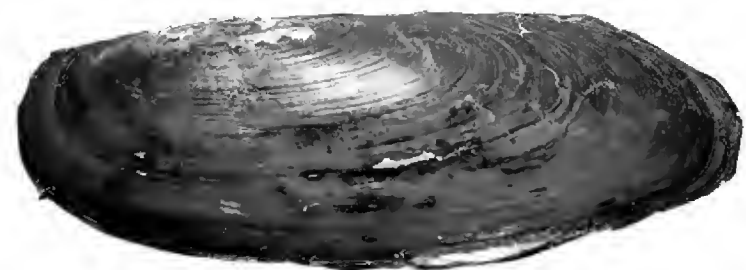


Fig.9



Fig.10



Fig.11

Figure 6. Native freshwater mussels *Anodontites patagonicus*, 70 mm (photo P. Lenhard).  
 Figure 7. Native freshwater mussel *Anodontites trapesialis*, 75 mm (photo P. Lenhard).  
 Figure 8. Native freshwater mussel *Leila blainvilleana*, 120 mm (photo P. Lenhard).  
 Figure 9. Native freshwater mussel *Mycetopoda legumen*, 85 mm (photo P. Lenhard).  
 Figures 10-11. Regional variations of the native freshwater mussel *Rhipidodonta charruana*, 30-35 mm (photo P. Lenhard / A.I. Agudo-Padrón).

*Rectartemon depressus* (Heynemann, 1868)  
Category IUCN: without category established

Recently included in the “Livro Vermelho da Fauna Brasileira Ameaçada de Extinção” (2003-2004).  
Class BIVALVIA

Order UNIONOIDA

Family MYCETOPODIDAE

*Anodontites elongatus* (Swainson, 1823)  
Category IUCN: without category established

Recently included in the “Livro Vermelho da Fauna Brasileira Ameaçada de Extinção” (2003-2004).

*Anodontites ferrarisi* (d’Orbigny, 1835)  
Category IUCN: in danger

*Anodontites patagonicus* (Lamarck, 1819) (Fig. 6)  
Category IUCN: in danger

*Anodontites tenebricosus* (Lea, 1834)  
Category IUCN: vulnerable

*Anodontites trapesialis* (Lamarck, 1819) (Fig. 7)  
Category IUCN: vulnerable

*Leila blainvilleana* (Lea, 1835) (Fig. 8)  
Category IUCN: in danger

*Mycetopoda legumen* (Martens, 1888) (Fig. 9)  
Category IUCN: vulnerable

*Mycetopoda siliquosa* (Spix, 1827)  
Category IUCN: vulnerable

Family HYRIIDAE

*Diplodon expansus* (Küster, 1856)  
Category IUCN: vulnerable

*Diplodon multistriatus* (Lea, 1834)  
Category IUCN: vulnerable

*Diplodon rhuacoicus* (d’Orbigny, 1835)  
Category IUCN: without category established  
Recently included in the “Livro Vermelho da Fauna Brasileira Ameaçada de Extinção” (2003-2004).

*Rhipidodonta charruana* (d’Orbigny, 1835) (Fig. 10)  
Category IUCN: vulnerable

Reported in the Brazilian lists (MMA, 2004; Agudo-Padrón, 2009c) under the taxonomic synonymy *Diplodon martensi* (Ihering, 1893) – see Simone (2006).

## CONCLUSIONS

The public seminar entitled “IV Fórum de Discussão sobre a Fauna ameaçada no Estado de Santa Catarina” and held in March 2010 concluded that the species considered in this study appear visibly undervalued in the Official listing compiled by regional environment institutions (IGNIS, 2010), with only a total listing of four related marine species (two bivalves = *Crassostrea brasiliiana*, *Euvola ziczac*; and two gastropods = *Hastula cinerea*, *Olivancillaria contortuplicata*).

It is hoped that soon this situation is properly reviewed, corrected and updated.

## ACKNOWLEDGEMENTS

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# Contribution to the Knowledge of longhorn beetles (Coleoptera, Cerambycidae) from Kenya

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**ABSTRACT** As a result of expeditions of the first author in Kenya during the period 2003-2006, 40 species and subspecies of longhorn beetles were collected and later determined by Dr. Karl Adlbauer. The faunistic list reports on recent nomenclature, localities of collection as well as geographical distribution of established taxa.

**KEY WORDS** Cerambycidae, longhorn beetles, Kenia.

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## INTRODUCTION

The cerambycid fauna of Ethiopian zoogeographical region includes over 3,000 valid species, but its actual number is undoubtedly greater due to the insufficient knowledge about longhorn beetles in the tropics and subtropics (Plavilstshikov, 1936). During the period 2003-2006, the first author collected many different insect species in Kenya. Among the other coleopterological material, a total number of 40 longhorn beetles were established. The main purpose of this note is to announce the species collected and to give some data about their distribution.

## MATERIALS AND METHODS

The study was conducted in different regions of Kenya during the period 2003-2006 (Figs. 1-6). The longhorn beetles were collected by traditional entomological methods:

- Hand collection of cerambycids on flowers and food plants (Fig. 7);
- Collection of cerambycids on grass and bushes by entomological bag;
- Shaking of tree branches and crowns and collection of fallen insects;
- Collection of cerambycids in sticky traps;

- Attracting cerambycids to lamp light;
- Rearing of adults in laboratory conditions from infested parts of food plants.

Collected cerambycids were identified by Dr. Karl Adlbauer.

Studied material is deposited in the Institute of Biodiversity and Ecosystem Research of Bulgarian Academy of Sciences Scientific Found (Sofia, Bulgaria). Single specimens are kept in K. Adlbauer's collection.

## RESULTS AND DISCUSSION

### PRIONINAE

#### Macrotomini

*Macrotoma palmata* (Fabricius, 1792)

Kenya, Elementeita Lake (00°28'31"S, 36°15'46"E), 1820 m, 14/15.IV.2006, 10 exx.; North-east Kenya, Lower Tana River, Gamba Guest House, sticky traps, 20/23.IV.2006, 1 ex.

Distribution: From Morocco to Saudi Arabia and RSA, Mauritius (Adlbauer et al., 2008).

*Prionotoma jordani* (Lameere, 1903)

Kenya, Bogoria Lake, 11.04.2004, 1 ex.

Distribution: From Senegal to Burundi and Angola (Delahaye et al., 2006).



Fig. 1



Fig. 2

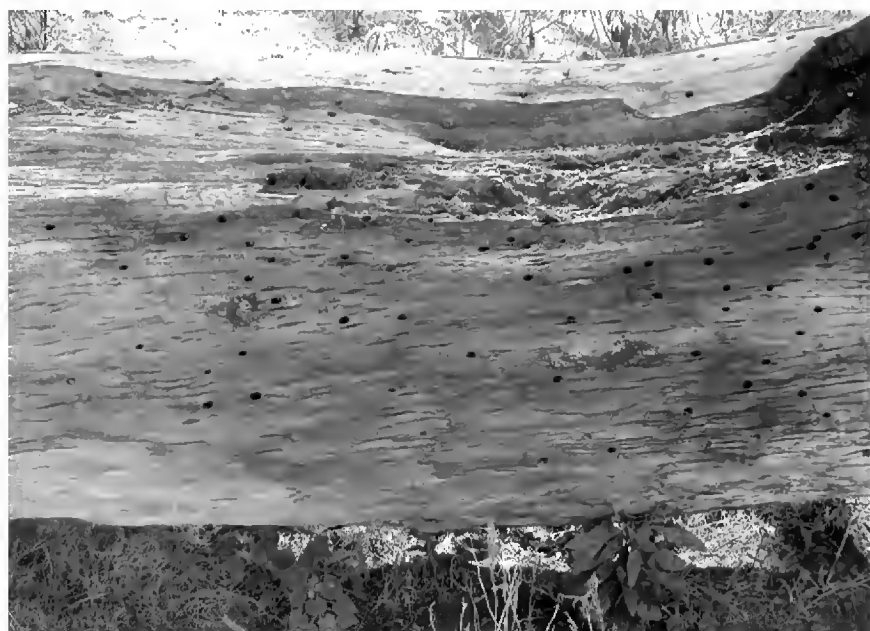


Fig. 3



Fig. 4



Fig. 5



Fig. 6

Figure 1. Kenia, Arabuko-Sokoke forest (photo Eduard Jendek).

Figure 2. Kenia, Elementeita lake (photo Eduard Jendek).

Figure 3. Kenia, dead trunk of *Acacia* sp. near Elementeita lake – habitat of *Macrotoma palmata* (photo Eduard Jendek).

Figure 4. Kenia, Taita hills forest (photo Eduard Jendek).

Figure 5. Tana river (photo Gianfranco Curletti).

Figure 6. Kenia, *Acacia lahai* forest in Ngong hills – favorite place for many coleopteran species (photo Eduard Jendek).

**CERAMBYCINAE****Xystrocerini**

*Xystrocera dispar* Fåhraeus, 1872

Kenya, Nyanza District, Ruma National Park, 1050 m, 04.VII.2003, 1 ex.; Kenya, Lower Tana River, Gamba, 25/27.X.2005, 3 exx.

Distribution: Tschad, Sudan, Saudi Arabia, Namibia and RSA (Adlbauer et al., 2008).

**Cerambycini**

*Neoplocaederus spinicornis* (Fabricius, 1781)

Kenya, Lower Tana River, Gamba Guest House, sticky traps, 20/23.IV.2006, 2 exx.

Distribution: Mauretania, Zimbabwe (Adlbauer et al., 2008).

**Molorchini**

*Merionoeda africana* Distant, 1899

NE Kenya, Arabuko – Sokoke Forest, 24/25.IV.2006, 1 ex.

Distribution: Congo-Kinshasa, Kenya, RSA (Adlbauer, 1995).

**Callichromatini**

*Litopus geniculatus* Harold, 1880

Kenya, Road Nairobi-Namanga, Nikobe, 1500 m, 04.XII.2003, 1 ex.

Distribution: Ethiopia, Tanzania (Adlbauer et al., 2008).

*Litopus kenyensis* Adlbauer, 2002

Kenya, Malindi, Kipepeo farm, (03°13'S, 40°06'E), 30 m, 24/25.VI.2006, 2 exx. (+ 1 ex. in K. Adlbauer's collection) (Adlbauer, 2002).

Distribution: Kenya.

*Paracolobizus bicolor* (Schmidt, 1922)

Kenya, Malindi, Kipepeo farm, (03°13'S, 40°06'E), 30 m, 24/25.VI.2006, 2 exx.

Distribution: Kenya, Tanzania (Juhel, 2010).

*Cloniophorus nyassae* (Bates, 1878)

Kenya, Shimba Hills, 150-200 m, 20.IV.2004, 1 ex.

Distribution: Kenya, Malawi (Schmidt, 1922).



Figure 7. *Oligosmerus* sp. (photo Eduard Jendek).

*Closteromerus claviger laevipes* Fairmaire, 1887

Kenya, Nairobi, 20.IV.2004, 6 exx.; Kenya, Ngong Hills Kiserian Distr. (01°26'56"S, 36°38'19"E), 1940 m, 17.IV.2006, 6 exx.

Distribution: Cameron, Eritrea, Somalia, Tanzania (Adlbauer et al., 2008).

*Rhopalomeces fulgurans* Schmidt, 1922

Kenya, Nyanza Province, Mbita, (0°24'S, 34°12'E), 1050 m, 18.V.2003, 1 ex.; Kenya, Rift Valley, Province N Kiserian, 1700 m, 10.VI.2003, 3 exx.; Kenya, Nairobi, 25.XI-20.XII.2003, 2 exx.; Kenya, Nairobi, 20.IV.2004, 3 exx.; Kenya, Narok, 1750 m, 12.V.2004, 1 ex.; Kenya, Nairobi, 10.V.2004, 1 ex.; Central Kenya, Elementeita Lake 1700 m, 12.V.2005, 1 ex.; Kenya, Ngong Hills, Kiserian Distr. (01°26'56"S, 36°38'19"E), 1940 m, 17.IV.2006, 11 exx.

Distribution: Tanzania (Schmidt, 1922).

*Rhopalomeces gracilis* (Fåhraeus, 1872)

NE Kenya, Arabuko – Sokoke Forest, 24/25.IV.2006, 1 ex.; Kenya, Malindi, Kipepeo farm (03°13'S, 40°06'E), 30 m, 24/25.VI.2006, 3 exx.

Distribution: Congo-Kinshasa, RSA (Adlbauer, 1995).

*Promeces longipes* (Olivier, 1795)

Kenya, Shimba Hills, 150-200 m, 20.IV.2004, 1 ex.

Distribution: Mocambique, RSA (Adlbauer, 2001).

*Promeces suturalis* (Harold, 1878)

Kenya, Gamba Distr., 14.IV.2006, 1 ex.; Kenya, Elementeita Lake (00°28'31"S, 36°15'46"E), 1820 m, 14/15.IV.2006, 1 ex.

Distribution: Kenya, Tanzania (Schmidt, 1922).

*Hypargyra albilateris* ssp. typ. (Harold, 1880)

Kenya, Rift Valley, Province N Kiserian, 1700 m, 10.VI.2003, 2 exx.; Kenya, Nairobi, 20.IV.2004, 1 ex.; Kenya, Ngong Hills, Kiserian Distr. (01°26'56"S, 36°38'19"E), 1940 m, 17.IV.2006, 4 exx.; NE Kenya, Malindi, Kipepeo Farm, 24.IV.2006, 1 ex.

Distribution: Ethiopia, Kenya, Tanzania (Juhel & Bentanachs, 2009).

**Clytini***Calanthemis subcruciatus* (White, 1855)

Kenya, Shimba Hills, 150-200 m, 20.IV.2004, 3 exx.; NE Kenya, Arabuko – Sokoke Forest, 24/25.IV.2006, 1 ex.

Distribution: Somalia, RSA (Adlbauer, 1995).

**LAMIINAE****Phantasini***Phantasis avernica* Thomson, 1865

Kenya, Elementeita Lake (00°28'31"S, 36°15'46"E), 1820 m, 14/15.IV.2006, 1 ex.

Distribution: Sudan, RSA (Sudre & Téocchi, 2000).

**Lamiini***Monochamus spectabilis* (Perroud, 1855)

NE Kenya, Malindi, Kipepeo Farm, 24.IV.2006, 1 ex.

Distribution: Ethiopia, Congo-Brazzaville, RSA, Madagascar, Comores (Adlbauer et al., 2008).

**Morimopsini***Monoxenus infraflavescens* Breuning, 1949

Kenya, Ngangao Forest, (03°21'59"S, 38°20'26"E), 1850 m, 4.XI.2005, 1 ex.

Distribution: Kenya (Breuning, 1950).

**Mesosini***Coptops aedificator* (Fabricius, 1792)

Kenya, Lower Tana River, Sailoni, 25.X.2005, 1 ex.

Distribution: Africa (including Seychelles, Comores, Madagascar), Saudi Arabia, SE-Asia, Hawaii (Adlbauer et al., 2008).

**Tragocephalini***Spilotragus guttatus* (Jordan, 1903)

Kenya, Road Nairobi-Namanga, Nikobe, 1500 m, 04.XII.2003, 1 ex.; Central Kenya, Road Kiserian to Oltepesi, 1770 m, 05.V.2005, 1 ex.

Distribution: Kenya (Breuning, 1934).

*Pseudochariesthes nigroguttata* (Aurivillius, 1908)

Central Kenya, Road Kiserian to Oltepesi, 1770 m, 05.V.2005, 1 ex.

Distribution: Kenya, Tanzania (Breuning, 1934).

**Prosopocerini***Prosopocera peeli* (Gahan, 1910)

Central Kenya, Elementeita Lake 1700 m, 12.V.2005, 1 ex.

Distribution: Ethiopia, Somalia, Kenya, Tanzania (Adlbauer et al., 2008).

**Ceroplesini, Subtribus Crossotina***Frema marmorata* Gerstaecker, 1871

NE Kenya, Arabuko – Sokoke Forest, ex. *Albicia* sp., 24/25.IV.2006, 1 ex.

Distribution: Kenya, Zimbabwe (Breuning, 1942).

*Frema aedificatoria* Hintz, 1910 = *Frema sublineata* Breuning, 1956

Kenya, Shimba Hills, 150-200 m, 20.IV.2004, 3 exx.

Distribution: Kenya, RSA (Breuning, 1942).

*Crossotus plumicornis* Serville, 1835

Kenya, Nyanza District, Ruma National Park, 1050 m, 04.VII.2003, 1 ex.; Kenya, Lower Tana River, ex. *Acacia* sp., 25.X.2005, 1 ex.

Distribution: Mauritania, RSA (Sudre et al., 2007).



*Crossotus barbatus* Gerstaecker, 1871

Kenya, Road Nairobi-Namanga, Nikobe, 1500 m, 04.XII.2003, 1 ex.

Distribution: Sudan, Somalia, Kenya, ?Malawi (Sudre et al., 2007).

**Ceroplesini, Subtribus Ceroplesina***Ceroplesis revoili pauli* Fairmaire, 1884

South Kenya, Jipe Lake Forest, ex. *Acacia* sp., 1 ex.

Distribution: Somalia, Kenya, Tanzania (Breuning, 1937).

*Ceroplesis bicincta* (Fabricius, 1798) (Fig. 8)

Kenya, Ngong Hills, Kiserian Distr. (01°26'56"S, 36°38'19"E), 1940 m, 17.IV.2006, 1 ex.

Distribution: Congo-Kinshasa, RSA (Adlbauer, 2001).

*Ceroplesis strandi* Breuning, 1935

Western Kenya, Narok, 1750 m, 12.V.2004, 1 ex.; Western Kenya, Narok, 1716 m, 18.V.2005, 2 exx. (+ 1 ex. in K. Adlbauer's collection).

Distribution: Zambia, Kenya (Breuning, 1937).

**Apomecynini***Enaretta caudata* (Fahraeus, 1872)

Central Kenya, Bogoria Lake, 900 m, 31.XI.2005, 1 ex.

Distribution: Uganda, RSA (Adlbauer, 2001).

**Eunidiini***Eunidia brunneopunctata strigatoides* Breuning, 1939

Kenya, Road Nairobi-Namanga, Nikobe, 1500 m, 04.XII.2003, 1 ex.; Kenya, Lower Tana River, Sailoni Forest (02°09'18"S, 40°11'04"E), 22/23.IV.2006, 1 ex.; Kenya, Road Voi to Taveta, Border of Tsavo West N. P. (03°30'10"S, 38°16'25"E), 28/30.IV.2006, 1 ex.

Distribution: Senegal, Ethiopia, RSA (Adlbauer et al., 2008).



Figure 8. *Ceroplesis bicincta* on *Acacia* sp. (photo Eduard Jendek)

**Pteropliini***Pterolophia variolosa* Kolbe, 1894

NE Kenya, Arabuko-Sokoke Forest, 24/25.IV.2006, 1 ex.

Distribution: Kenya, Tanzania (Breuning, 1961a).

**Saperdini***Glenea apicalis westermanni* (Thomson, 1860)

Kenya, Gilgil Distr., 14.IV.2006, 1 ex.

Distribution: Togo, RSA (Adlbauer, 2001).

*Glenea arida* Thomson, 1865

NE Kenya, Arabuko-Sokoke Forest, 24/25.IV.2006, 1 ex.

Distribution: Kenya, RSA (Breuning, 1958).

*Phytoecia (Plepisanis) neavei* Aurivillius, 1914

Kenya, Narok, 1750 m, 12.V.2004, 1 ex.

Distribution: Kongo-Kinshasa, Uganda, Malawi (Breuning, 1951).

*Phytoecia (Blepisanis) suturevittata* Breuning, 1951

NE Kenya, Arabuko-Sokoke Forest, 24/25.IV.2006, 2 exx. (+ 1 ex. in K. Adlbauer's collection).

Distribution: Kenya (Breuning, 1951).

*Phytoecia (Pseudoplepisanis) somereni* Breuning, 1951

Kenya, Narok, 1750 m, 12.V.2004, 1 ex.

Distribution: Kenya (Breuning, 1951).

*Oberea pagana* Harold, 1880

Central Kenya, Road Kiserian to Oltepesi, 1750-1770 m, 05.V.2005, 1 ex.

Distribution: Ethiopia, Kenya (Adlbauer et al., 2008).

*Oberea cingulata* Aurivillius, 1914

Kenya, Ngong Hills, Kiserian Distr., (01°26'56"S, 36°38'19"E), 1940 m, 17.IV.2006, 1 ex.

Distribution: Lake Victoria, Kenya, Tanzania (Breuning, 1961b).

## CONCLUSIONS

Four species found in this study are known with limited distribution only in Kenya – *Monoxenus infraflvenscens*, *Spilotragus guttatus*, *Phytoecia suturevittata* and *P. somereni* – which make them potential endemics for this country. Other five cerambycids appear to be most probably new for Kenya: *Prionotoma jordani*, *Rhopalomeces fulgurans*, *Rhopalomeces gracilis*, *Promeces longipes* and *Ceroplesis bicincta*. It could be noted that new records enlarge our knowledge on these species distribution and increase species diversity of Kenyan fauna. As a main conclusion we have to underline that the Kenyan longhorn beetles fauna is partially and incompletely studied that is why any new contribution is very important to enrich our learning of this fauna.

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## Genetic diversity analysis of the durum wheat Graziella Ra, *Triticum turgidum* L. subsp. *durum* (Desf.) Husn. (Poales, Poaceae)

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**ABSTRACT** For the first time, the durum wheat Graziella Ra was compared to four Italian durum wheat varieties (Cappelli, Grazia, Flaminio and Svevo) and to Kamut in order to preliminary characterize its genome and to investigate genetic diversity among and within the accessions by Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs) and  $\alpha$ -gliadin gene sequence analysis. The main aim of the study was an attempt to determine the relationship between the historic accession Graziella Ra and Kamut which is considered an ancient relative of the durum subspecies. In addition, nutritional factors of Graziella Ra were reported. Obtained results showed that (i) both AFLP and SSR molecular markers detected highly congruent patterns of genetic diversity among the accessions showing nearly similar efficiency; (ii) for AFLPs, percentage of polymorphic loci within accession ranged from 6.57% to 19.71% (mean 12.77%) and, for SSRs, from 0% to 57.14% (mean 28.57%); (iii) principal component analysis (PCA) of genetic distance among accessions showed the first two axes accounting for 58.03% (for AFLPs) and 61.60% (for SSRs) of the total variability; (iv) for AFLPs, molecular variance was partitioned into 80% (variance among accessions) and 20% (within accession) and, for SSRs, into 73% (variance among accessions) and 27% (within accession); (v) cluster analysis of AFLP and SSR datasets displayed Graziella Ra and Kamut into the same cluster; and (vi) molecular comparison of  $\alpha$ -gliadin gene sequences showed Graziella Ra and Kamut in separate clusters. All these findings indicate that Graziella Ra, although being very similar to Kamut, at least in the little part of the genome herein investigated by molecular markers, may be considered a distinct accession showing appreciable levels of genetic diversity and medium-high nutritional qualities.

**KEY WORDS** AFLP,  $\alpha$ -gliadin gene, durum wheat; genetic diversity analysis, nutritional qualities, SSR; *Triticum*.

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### INTRODUCTION

Durum wheat (*Triticum turgidum* L. subsp. *durum*) is the only tetraploid (AABB,  $2n=4x=28$ ) species of wheat of commercial importance that is widely cultivated today. It originated thousands of years ago from a hybridization (pollen exchange) of the wild diploid *T. monococcum* L. (A genome) and the donor of the B genome which, according to morphological, geographical and cytological evidence, has recently been recognized as *T. speltoides* (Tausch) Gren. or a closely related species (von Buren, 2001). In the last decades, a huge number of durum wheat cultivars have been obtained by

artificial selection, generally based on high yield, disease resistance and technological qualities (e.g. bread- or pasta-making qualities) with little emphasis on taste or dietary components. On the other hand, at the same time, traditional local varieties have been considerably reduced as a result of the diffusion of new varieties of wheat. To preserve genetic variability and reduce genetic erosion it is extremely important developing and maintaining local collections, including old cultivars and landraces, which – at least in some cases – may be employed for niche cereal-based typical products. This was the case of Graziella Ra, an ancient accession (not a cultivar) of durum wheat which, thanks to its

good taste and fine pasta-making qualities, recently appeared on the market as Graziella Ra®, an Italian trademark used in marketing products made with the homonymous grain. Currently, it is organically grown in Marche (central Italy) by Alce Nero Cooperative (Urbino, PU) mainly with the aims to contribute to the preservation of local biodiversity and increase the interest for ancient crops which are at the basis of the Mediterranean diet.

This study was designed with the intent of providing a preliminary characterization of Graziella Ra genome, analysed for the first time. To this aim, other five accessions chosen as representatives of modern (Grazia, Flaminio, Svevo), traditional (Cappelli) and ancient (Graziella Ra, Kamut) wheats were selected to obtain a small set of three modern and three older durum accessions. Comparative analysis was carried out by AFLPs (Amplified Fragment Length Polymorphisms), microsatellites (SSRs, Simple Sequence Repeats) and the  $\alpha$ -gliadin gene sequence to evaluate genetic diversity within and among wheats under study.

## MATERIALS AND METHODS

### Accessions

Graziella Ra (Fig. 1a) is a type of durum wheat characterized by low yield (15-20 quintals per hectare), medium-long cycle, tall size (about 120 cm) and a phenotype very similar to Kamut's (see below) with large ears and long aristas. It was brought to Italy at the end of '70s (see <http://www.alcenerocooperativa.it/pagina.asp?pag=443>), forgotten for a long time and rediscovered a few years ago due to its fine pasta-making qualities. Cappelli is an Italian traditional strain of durum wheat which deserves a privileged place among the varieties of old established durum wheat for being the very first selected variety. Svevo, Grazia and Flaminio are modern cultivars, with a great commercial importance, employed for pasta or bread-making. Kamut is a registered trademark of Kamut International, Ltd., used in marketing products made with the variety QK-77. It is characterized by erect young shoots with very narrow pubescent leaves, the plants tiller very little and the straw thin. The spikes are narrow, lax or very

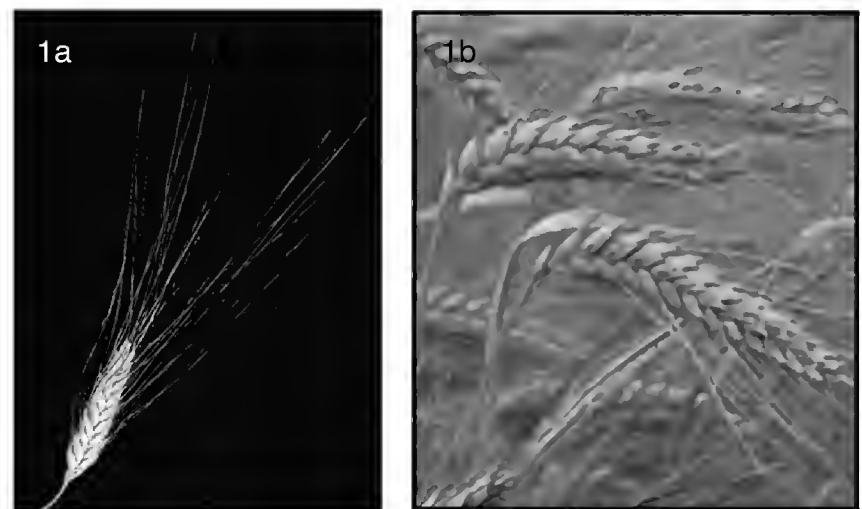


Figure 1. Spike morphology of Graziella Ra (1a) and Kamut (1b) durum wheats.

lax with long narrow white glumes. The spikelet lemmas have long and strong, more or less deciduous, white or black awns (Fig. 1b). The grains are very large - up to twice the size of bread wheat kernels - narrow, vitreous, and flinty with a characteristic hump. The correct subspecies is still in dispute; in fact, according to Stallknecht et al. (1996), Kamut has been classified, from time to time, as *T. turgidum polonicum*, *T. turgidum turanicum* or *T. turgidum durum*. Although its taxonomy is contentious, it is considered an ancient relative of durum subspecies. All wheats were provided by Alce Nero Cooperative, with the exception of Kamut, kindly supplied by Molini del Conero (Osimo, AN, Italy).

### DNA extraction

Several seeds of each line were germinated in the dark for two days. The seedlings were grown in daylight for seven days. Leaf tissues - sampled at the four-leaf stage from twenty different plants per accession - were immediately frozen in liquid nitrogen and ground in a mortar with a pestle. Thirty mg of powder were used for DNA extraction following the cetyltrimethylammonium bromide (CTAB) protocol (Doyle & Doyle, 1990) with slight modifications. DNA quality was tested by a 0.8% agarose gel electrophoresis.

### AFLP

AFLP genotyping was performed at Keygene NV (Wageningen, The Netherlands) using their standard in-house developed protocols (Vos et al., 1995). Briefly, DNA extracted from four different plants for each parental line (for a total



of twenty-four samples) was fingerprinted using ten AFLP primers, five *Pst*I (indicated as P35, P36, P39, P41, P42) and five *Taq*I (T40, T41, T42, T44, T46) (Table 1) arranged in eight primer combinations (P35/T44; P35/T46; P36/T46; P39/T41; P39/T42; P41/T40; P41/T41 and P42/T41) (Table 2).

### Microsatellite genotyping

Twelve different plants per accession (seventy-two individuals) were employed. Nine Simple Sequence Repeat (SSR) markers were selected from several ones tested on the grounds of their T<sub>m</sub>, length and degree of polymorphism. Primers are listed in Table 3. A tailed PCR primer was used for SSR analysis by adding a 19-base M13 oligo sequence (M13 tail) to the 5' end of each forward SSR primer. Thus, each SSR reaction used three primers: two unlabelled SSR primers one of which having an attached M13 sequence tail (5'-CACGACGTTGTAAAACGAC-3'), and one universal FAM-labelled M13 primer with the same sequence as the M13 tail (Schuelke, 2000; Boutin-Ganache et al., 2001; Fukatsu et al., 2005). PCR reactions were carried out in 10 µl of a solution containing 10 ng genomic DNA, 1x Mg-free PCR buffer solution, 0.25 mM dNTPs, 1.5 mM MgCl<sub>2</sub>, 50 nM forward primer, 5.0 nM reverse primer, 500 nM M13-labelled primer, 0.5 U AmpliTaq Gold DNA polymerase (Applied Biosystems) and nuclease-free water. Amplification was performed as follows: 5 min at 95 °C; 20 sec at 94 °C, 30 sec at 55 °C, 30 sec at 72 °C (42 cycles); and a final extension stage of 5 min at 72 °C. PCR products were separated with an ABI 3730 DNA sequencer (Applied Biosystems) and the fragments were sized by means of a ladder labelled with a fluorochrome VIZ (LIZ500, Applied Biosystems). Data were analysed with GeneMapper 3.0 (Applied Biosystems).

### *A-gliadin gene Amplification, Cloning, Sequencing and Analysis*

DNA from two plants per accession (total of twelve samples, different from samples employed for molecular markers) was used for PCR amplifications of the  $\alpha$ -gliadin gene. Both forward (5'-ATGAAGACCTTTCTCATCC-3')

and reverse (5'-YYAGTTRGTACCGAAGATGCC-3') primers were designed on the conserved 5' and 3' ends of the coding region of the  $\alpha$ -gliadin gene sequences downloaded from the GenBank database (ID: DQ296195, DQ296196 and AJ870965). PCR amplifications were carried out - using a high fidelity Pfu DNA Polymerase (Promega) - as follows: 95 °C for 2 min; 95 °C for 1 min, 60 °C for 30 sec, 72 °C for 2 min (30 cycles); 72 °C for 5 min. Reaction products were visualized by electrophoresis on a 1.2% agarose gel containing TBE 1X buffer and ethidium bromide (0.5 µg/ml). An aliquot (1 µl) of the PCR product was inserted into a pCR 4-TOPO vector by the TA-cloning system and transformation was performed on *E. coli* TOP10 cells following the manufacturer's instructions (Invitrogen). Selected transformants were analysed for presence of the insert by PCR, grown in LB medium overnight and purified by the Wizard Plus SV minipreps kit (Promega). Finally, sequencing of plasmid inserts was done by using automated DNA sequencers at Eurofins MWG Operon. Sequences were visualized with BioEdit Sequence Alignment Editor 7 (Hall, 1999), aligned with the ClustalW option included in this software and double checked by eye. Standard measures of nucleotide polymorphism [mean pairwise differences ( $k$ ), nucleotide diversity ( $\pi = P_i$  and  $\pi_{JC} = P_i$  corrected according to Jukes and Cantor) and nucleotide divergence ( $D_{xy}$ ) between accessions] using the full set of all sequences were computed by DNAsp 5 (Librado & Rozas, 2009).

### Statistical analysis

For AFLP and SSR datasets, analyses were performed within GenAlEx 6.4 (Peakall & Smouse, 2006), a user-friendly package with an intuitive and consistent interface that allows to analyse a wide range of population genetic data, including both dominant (AFLP) and codominant (SSR) datasets, within MS Excel. For each accession, allele number ( $N_a$ ), heterozygosity ( $H_e$ ), number and frequency of genotypes and percentages of polymorphic loci were obtained by the software. Polymorphism information content (PIC) of each SSR was computed according to Botstein et al. (1980). Nei's unbiased genetic distance (Nei, 1978) was

<i>Primer name</i>	<i>sequence</i>
P35	5'-GACTGCGTACATGCAG ACA-3'
P36	5'-GACTGCGTACATGCAG ACC-3'
P39	5'-GACTGCGTACATGCAG AGA-3'
P41	5'-GACTGCGTACATGCAG AGG-3'
P42	5'-GACTGCGTACATGCAG AGT-3'
T40	5'-GATGAGTCCTGACCGA AGC-3'
T41	5'-GATGAGTCCTGACCGA AGG-3'
T42	5'-GATGAGTCCTGACCGA AGT-3'
T44	5'-GATGAGTCCTGACCGA ATC-3'
T46	5-GATGAGTCCTGACCGA ATT-3'

Table 1. *Pst*I (P) and *Taq*I (T) primers employed for AFLP analysis

Primer combinations	No. of polymorphic bands	Mean diversity index (He)	Marker index*
P35/T44	19	0.082	1.56
P35/T46	11	0.018	0.20
P36/T46	16	0.087	1.39
P39/T41	23	0.044	1.01
P39/T42	13	0.003	0.04
P41/T40	17	0.010	0.17
P41/T41	13	0.029	0.38
P42/T41	25	0.032	0.80
*MI = (no. of polymorphic loci/PC) x (mean diversity index/PC); for details, see Powell et al. (1996).			

Table 2. Polymorphism features of the eight AFLP primer combinations (PCs) used to estimate genetic similarities among wheat accessions under study.

<i>SSR</i>	<i>Primer sequence</i>
Barc174	For 5' - TGGCATT TTTCTAGCACCAATACAT Rev 5' - GCGAACTGGACCAGCCTTCTATCTGTTC
DuPw217	For 5' -CGAATTACACTTCCTTCTTCCG Rev 5' -CGAGCGTGTCTAACAAGTGC
Xgwm750	For 5' - CTTGCACAGAGACGATGCAT Rev 5'-TGAGTCAGTCTCACAACCGG
Xgwm1045	For 5' - ATCACAAGGAGTTTATCGCT Rev 5' - GTCAATGGACCATGGGATTC
Xgwm1038	For 5' - GTGCTCCATGGCGTCTG Rev 5' - AGTCCAGCAAACATTCTCCA
Xgwm126	For 5' - CACACGCTCCACCATGAC Rev 5' - GTTGAGTTGATGCGGGAGG
Xgwm1027	For 5' - CAGTTCTCCCGGCATGTATT Rev 5' - TTCACATTGTCGCGTTGAAT

Table 3. List of primers used for SSR analysis

calculated in the TFPGA program (Miller, 1997). For both AFLPs and SSRs, phenetic diagrams were constructed on corresponding pairwise genetic distance matrices by the Unweighted Pair-Group Method using Arithmetic averages (UPGMA) (Sneath & Sokal, 1973) with the UPGMA tree searching algorithm of the software. A thousand replicate distance matrices were bootstrapped to evaluate the robustness of the trees. For both datasets, analysis of molecular variance (AMOVA) was carried out to examine total genetic variation among and within accessions; in addition, Principal Component Analysis (PCA) was performed in order to more effectively view the patterns of genetic distance. A Mantel test was used to detect the possible correlation between AFLP and SSR accession matrices. Statistical significance was determined by random permutations, with the number of permutations set to 9,999.

### ***Phylogenetic analysis***

Phylogenetic analyses were conducted in MEGA 5 (Tamura et al., 2011) and BEAST 1.4.8 (Drummond & Rambaut, 2007) by Maximum Likelihood (ML) and Bayesian Inference (BI). For maximum likelihood analyses, the most appropriate model of DNA substitution resulted HKY (Hasegawa Kishino Yano). Bayesian analysis was conducted by BEAST where the topology and divergence times can be estimated simultaneously from the data and therefore a starting tree topology is not required, making it particularly appropriate for groups with uncertain phylogenies. BEAST input files were generated with BEAUTi (v 1.4.8) using the  $\alpha$ -gliadin gene dataset (nexus format) and a HKY substitution model. For partition into codon positions, the SRD06 model (Shapiro et al., 2006) was selected; this model links 1st and 2nd codon positions but allows the 3rd positions to have a different relative rate of substitution, transition-transversion ratio and gamma distributed rate heterogeneity and has been found to provide a better fit for protein-coding nucleotide data. BEAST was run for 1,000,000 generations with samples taken every 100 generations. Five independent Markov Chain Monte Carlo (MCMC) runs were conducted and the log and tree files were combined using

LogCombiner (v 1.4.8). The results were examined by Tracer (v 1.5) to confirm stationary distribution and adequate effective sample sizes (i.e. ESS>200) for all parameters, indicating that the sampled generations were uncorrelated and the posterior distribution of the parameter was long and accurate. TreeAnnotator (v 1.4.8) was then used to summarize a best supported tree and annotate the tree with posterior probabilities of the nodes under investigation. FigTree (v 1.3.1) was used to display the 95% confidence intervals. BEAST, BEAUTi, LogCombiner, Tracer, TreeAnnotator and FigTree were downloaded from <http://beast.bio.edu.ac.uk>.

Support for the internodes was assessed by bootstrap percentages (100 replicates for ML), whereas for Bayesian inference tree, the Bayesian posterior probability was computed.  $\alpha$ -gliadin gene sequences from *Triticum aestivum* L. (GenBank ID: DQ166377) and *T. dicoccoides* Korn. (GenBank ID: DQ140352) were employed as outgroups.

### ***Nutritional quality***

*Graziella Ra* was investigated by Eurofins Biolab srl (an Italian company specialized in assays and controls, and in biological, microbiological and chemical determinations) using their standard in-house developed protocols; each analysis was made in triplicates. For Kamut, we report nutritional values available at <http://www.kamut.com>.

## **RESULTS AND DISCUSSION**

### ***Molecular marker variation***

A total of twenty-four individuals were investigated using eight AFLP primer combinations. One sample (from Svevo) didn't generate reliable fingerprintings and was excluded from the analysis which, therefore, resulted in twenty-three individuals showing a total of 137 markers. For each AFLP primer combination, number of polymorphic bands, mean heterozygosity and marker index are reported in Table 2. The presence/absence of each fragment was encoded as a 1/0 score, generating a binary data matrix. Within each accession, mean heterozygosity  $\pm$  standard error

SSR	Chromosome	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5	Allele 6	PIC	Mean He	MI*
Xgwm126	5A	203	206	212	214	-		0.60	0.111	0.44
Barc174	1B	200	201	216	-	-		0.45	0.030	0.09
Xgwm1045	2A	192	198	202	-	-		0.61	0.058	0.17
Xgwm1038	3A	235	241	242	252	254	274	0.35	0.072	0.43
Xgwm750	1A	230	234	236	249	-		0.68	0.102	0.41
Xgwm1027	2B	125	129	138	-	-		0.67	0.074	0.22
DuPw217	6B	232	241	242	-	-		0.30	0.025	0.07

MI = (no. of polymorphic bands/SSR) x (mean diversity index/SSR); for details see Powell et al. (1996).

Table 4. List of all the alleles revealed by the microsatellites in the six accessions. Chromosome mapping, PIC, Polymorphism Information Content; He, heterozygosity (also called diversity index); MI, Marker index are reported. Please note that alleles are expressed in nucleotide length (bp = base pairs).

and percentage of polymorphism resulted specifically:  $0.024 \pm 0.008$ , 6.57% (Svevo);  $0.081 \pm 0.014$ , 22.63% (Flaminio);  $0.027 \pm 0.008$ , 8.03% (Kamut);  $0.051 \pm 0.012$ , 11.68% (Graziella Ra);  $0.034 \pm 0.010$ , 8.03% (Cappelli); and  $0.083 \pm 0.015$ , 19.71% (Grazia). Percentage of polymorphism was, on average, 12.77%.

SSR data were classified according to a qualitative scale, with scores ranging from 1 to 5, describing the complexity of the amplification profile for each primer (Stephenson et al., 1998). Out of nine markers considered, seven [Barc174, Xgwm750, Xgwm1038, Xgwm126 and Xgwm1027 (score 1, 2); Xgwm1045 and DuPw217 (score 3)] were included in the analysis; whereas two (Xgwm1136 and Xgwm1009) failed to give rise to any amplification products. SSRs revealed a total of 26 alleles in the six accessions. The number of alleles per locus varied among these markers, ranging from three (DuPw217, Barc174, Xgwm1027, Xgwm1045) to six (Xgwm1038) with an average of 3.7. As a measure of the informativeness of microsatellites, the average PIC (Polymorphism Information Content) value was 0.53, ranging from 0.30 (DuPw217) to 0.68 (Xgwm750). For each marker, number of alleles, PIC value, mean heterozygosity and marker index (MI), a universal metric to represent the amount of information obtained per experiment, are reported in Table 4. As shown, marker index values are not very high but, on the other hand, considering that a PIC value  $> 0.5$  accounts for a highly informative marker,  $0.5 > PIC > 0.25$  for

Accession	SSR	Allele (in bp)	Freq (%)
Svevo	Xgwm126	203	66.7
Svevo	Xgwm126	212	16.7
<b>Svevo</b>	<b>Xgwm1038</b>	<b>235</b>	<b>4.2</b>
Svevo	Xgwm1038	252	95.8
Flaminio	Barc174	216	10
Graziella Ra	Xgwm750	249	9.1
Cappelli	Xgwm750	236	33.3
Cappelli	DuPw217	242	8.3
<b>Grazia</b>	<b>Xgwm1038</b>	<b>241</b>	<b>4.2</b>
Grazia	Xgwm1038	254	12.5
<b>Grazia</b>	<b>Xgwm1038</b>	<b>274</b>	<b>4.2</b>
<b>Grazia</b>	<b>DuPw217</b>	<b>232</b>	<b>100</b>

Table 5. Unique alleles observed by SSR molecular markers. Rare (frequency  $< 5\%$ ) and diagnostic alleles (frequency = 100%) are in bold.

an informative marker, and  $PIC \leq 0.25$  for a slightly informative marker (Botstein et al., 1980), PIC values suggest that SSRs employed in the present study resulted adequate and efficient. With reference to the percentage of polymorphism within each accession, observed values ranged between 0% (Kamut) and 57.14% (Svevo and Graziella Ra), going through 14.29% (Flaminio and Grazia) and 28.87% (Cappelli), with an average value of 28.57%. Based on SSR markers herein reported along with the limited number of accessions under investigation, Table 5 summarizes private alleles observed in this study, which may be used as a simple indirect measure



of genetic diversity. As shown, Grazia and Svevo have the greatest number (four) of accession-specific alleles; moreover, in Grazia the 232 bp allele is monomorphic and hence could be considered as diagnostic for the identification of the variety; private alleles were observed in nearly all the accessions, though three of them were rare, with a percentage below 5%.

Average heterozygosities for AFLPs and SSRs were not significantly different (*t* test,  $p > 0.05$ ).

### Cluster analysis

Genetic distance was calculated using Nei's index. Cluster analysis applied to genetic distance matrices produced the phenetic diagrams shown in figures 2a and 2b. In both cases, Kamut and Graziella Ra resulted very similar.

For both AFLPs and SSRs, patterns of PCA revealed by the first two principal coordinate axes accounted for the most of the variation in the data, and so only the first two dimensions were plotted in this paper. With reference to pairwise individual genetic distance matrices, the first two axes accounted for 63.15% (38.56% and 24.59%) of the AFLPs and 64.42% (42.26% and 22.16%) of the SSRs variation (Figs. 3a and 3b); taking into account PCA of genetic distances among accessions, the first two axes explained 58.03% (34.95% and 23.08%) of the AFLPs and 61.60% (36.11% and 25.49%) of the SSRs variation. As shown in figures 3c and 3d, a high degree of similarity between Graziella Ra and Kamut was confirmed also by PCA.

### Analysis of molecular variance (AMOVA)

Analysis of molecular variance partitioned the total genetic variance into variance among populations and within population. For AFLPs, total variance was partitioned into 80% (variance among populations) and 20% (within population) (Fig. 4a); for SSRs, into 73% (among populations) and 27% (within population) (Fig. 4b).

### Correlation between AFLPs and SSRs

A strong correlation ( $r^2 = 0.92$ ) between AFLP and SSR population data matrices was obtained by the Mantel test (Fig. 5). This finding suggests that both types of molecular markers detected

highly congruent patterns of genetic diversity, at the accession level, showing nearly similar efficiency. In fact, AFLP and SSR average heterozygosities were not significantly different and observed values of MI or polymorphism levels were in line with distinctive nature of these markers. In particular, a higher MI for AFLPs (0.69 vs. 0.26) was the result of a higher multiplex ratio component, due to the simultaneous detection of several polymorphic markers per single reaction. On the contrary, a lower number of total bands was obtained for SSRs, but all of these were polymorphic, thus giving a higher average percentage of polymorphism (28.57% vs. 12.77%) and providing higher genetic diversity within a given accession and lower genetic differentiation among accessions than AFLP markers, which was confirmed by AMOVA results as well.

### A-gliadin gene

A-gliadin is a very important storage protein widely studied for its implication in coeliac disease (i.e. Koning, 2005; Gregorini et al., 2009 and references therein). In this study molecular analysis of the  $\alpha$ -gliadin gene sequence was employed either to analyse diversity at the gene level or to provide a possible reconstruction of phylogenetic relationships among wheats under study.

A-gliadin gene complete sequences obtained in this study are available at GenBank as GQ999807 (Cappelli, 903 bp), GQ999809 (Flaminio, 942 bp), GQ999811 (Grazia, 963 bp), GQ999813 (Graziella Ra, 909 bp), GQ999815 (Kamut, 942 bp), GQ999817 (Svevo, 942 bp). Sequences alignment showed 78 variable sites, 79 mutations ( $S = 78$ ,  $Eta = 79$ ) and 75 insertions/deletions. Nucleotide diversity ( $\pi$ ) was  $0.032 \pm 0.011$  and  $0.033$  when corrected according to Jukes and Cantor ( $\pi_{JC}$ ). The average number of nucleotide differences ( $k$ ) was 28.867. Assessed mean sequence identity was 91.5%; in particular,  $\alpha$ -gliadin genes from Graziella Ra and Kamut were 95% identical. Deduced  $\alpha$ -gliadin protein sequences showed a mean identity of 89.4%;  $\alpha$ -gliadins from Graziella Ra and Kamut were 94.3% identical. Maximum likelihood and Bayesian Inference phylogenetic reconstructions produced nearly identical results. ML and BI

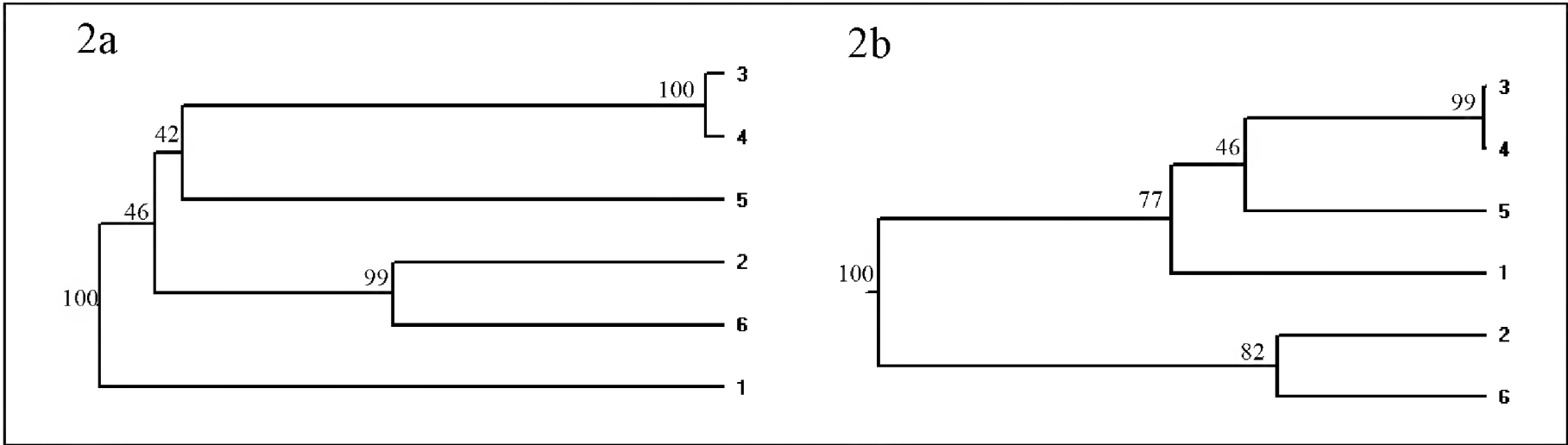


Figure 2. 2a. Dendrograms of the six wheat accessions based on Nei's genetic distance calculated using 137 amplified fragment length polymorphisms (AFLPs); 2b. Dendrograms of the six wheat accessions based on Nei's genetic distance calculated using seven simple sequence repeats (SSRs). 1, Svevo; 2, Flaminio; 3, Kamut; 4, Graziella Ra; 5, Cappelli; 6, Grazia. Bootstrap supporting values (1,000 replicates) are reported on the nodes.

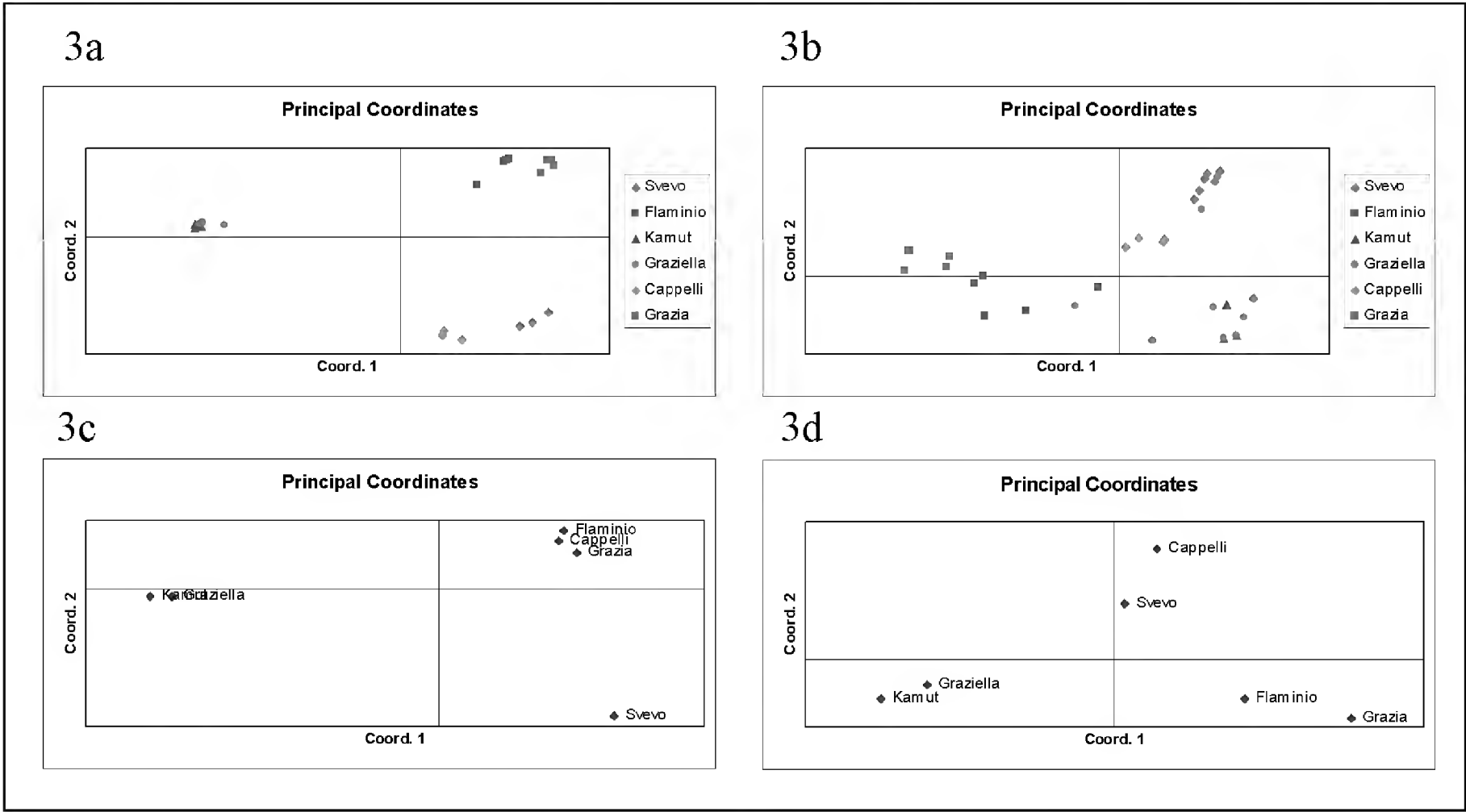


Figure 3. Principal Component Analysis (PCA) plots of the first two axes based on genetic distance matrices among individuals for AFLP (3a) and SSR (3b) datasets; PCA plots of the first two axes based on genetic distance matrices among accessions for AFLP (3c) and SSR (3d) datasets.

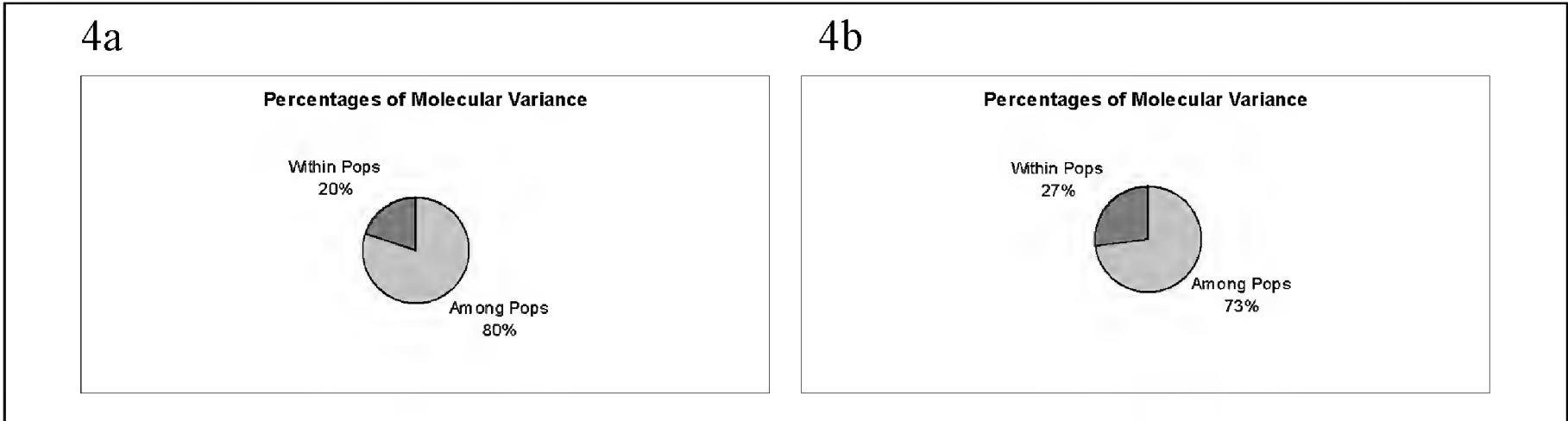


Figure 4. Results of Analysis of Molecular variance (AMOVA) for the total AFLPs (4a) and SSRs (4b) showing the percentage of variation among and within accessions.

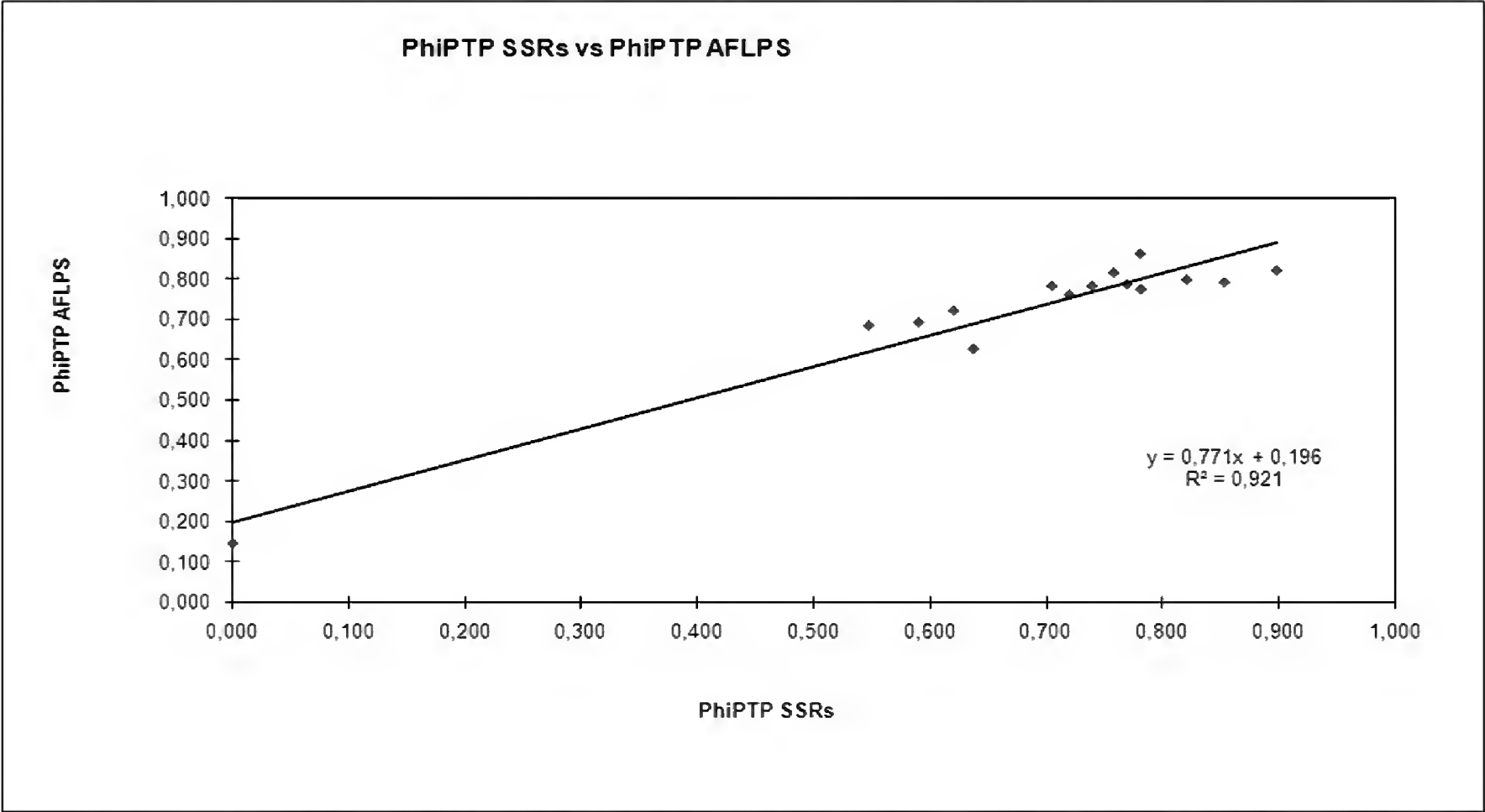


Figure 5. Output of Mantel test comparing the AFLP and SSR genetic distance matrices at accession level.

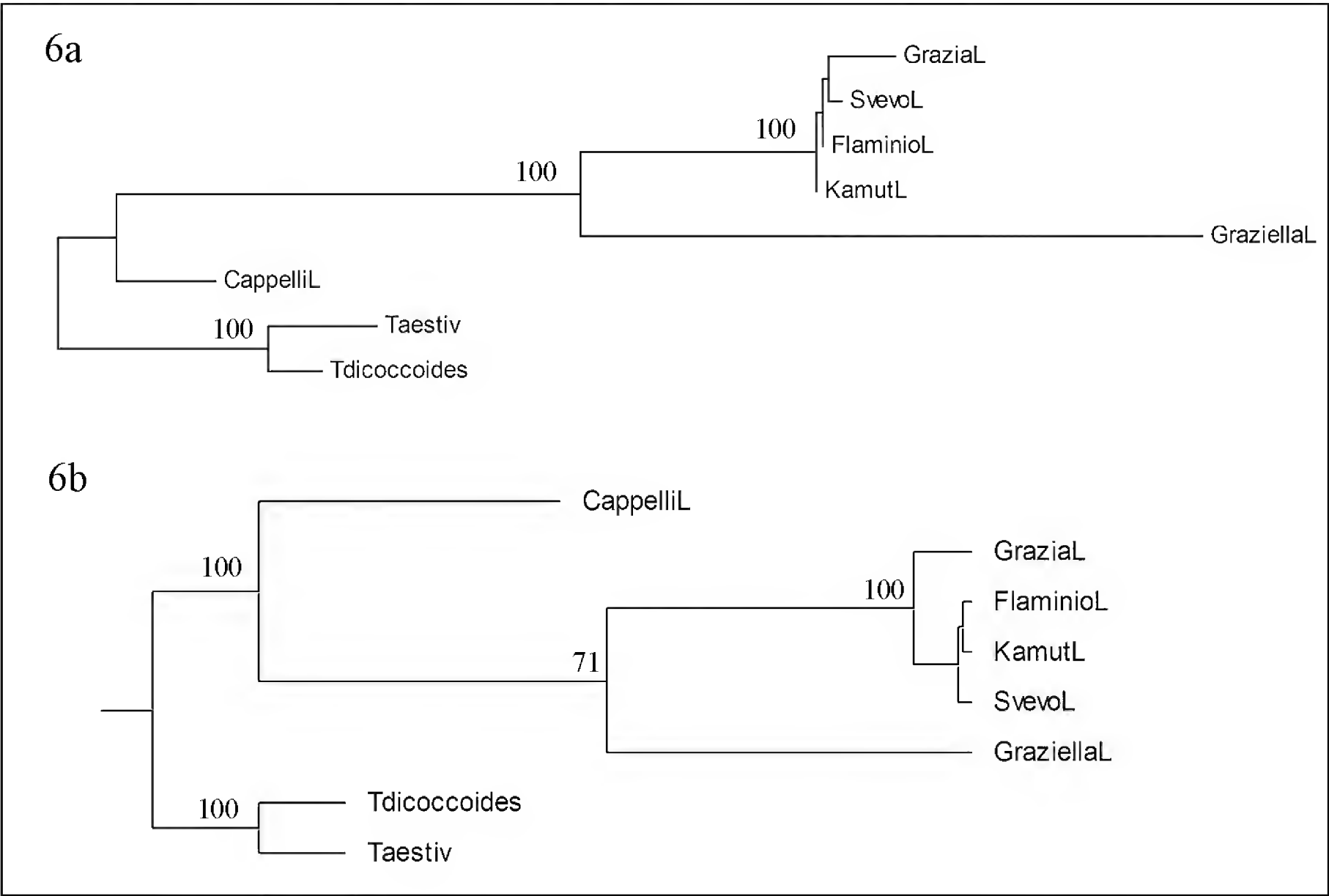


Figure 6. 6a. 50% majority rule Maximum Likelihood consensus tree inferred from the  $\alpha$ -gliadin gene sequence alignment. Numbers above branches represent bootstrap values (100 replicates). 6b. Bayesian consensus tree inferred from the  $\alpha$ -gliadin gene sequence alignment. Numbers above branches represent Bayesian posterior probabilities. *T. aestivum* and *T. dicoccoides* were employed as outgroups to root the trees.

	<i>Common wheat*</i>	<i>Kamut</i>	<i>Graziella Ra</i>
Water	11.5%	9.8%	10.8%
Protein**	14%	19.6%	11.80%
Total lipid (fat)	1.9%	2.6%	2.91%
Carbohydrate	72.7%	68.2%	61.23%
Crude fiber	2.1%	1.8%	2.7%
Ash	1.66%	1.82%	2.02%
MINERALS (mg/100g)			
Calcium	30	31	31.2
Iron	3.9	4.2	2.5
Magnesium	117	153	85.3
Phosphorus	396	411	450
Potassium	400	446	379.1
Sodium	2.0	3.8	5.8
Zinc	3.2	4.3	38
Copper	0.44	0.46	0.5
Manganese	3.8	3.2	2.2
Selenium (mg/kg)		1.6-7	2
VITAMINS (mg/100g)			
Thiamine (B1)	0.42	0.45	>0.05
Riboflavin (B2)	0.11	0.12	0.02
Niacin	5.31	5.54	7.83
Panthenic acid	0.91	0.23	0.04
Vitamin B6	0.35	0.08	0.94
Folacin	0.0405	0.0375	0.031
Vitamin E	1.2	1.7	0.43
AMINOACIDS (g/100g)			
Tryptophan	0.194	0.117	-
Threonine	0.403	0.540	0.42
Isoleucine	0.630	0.600	0.78
Leucine	0.964	1.23	0.86
Lysine	0.361	0.440	0.34
Methionine	0.222	0.250	-
Cystine	0.348	0.58	-
Phenylalanine	0.675	0.85	0.36
Tyrosine	0.404	0.430	0.21
Valine	0.624	0.800	0.46
Arginine	0.610	0.860	0.69
Histidine	0.321	0.430	0.29
Alanine	0.491	0.630	0.45
Aspartic acid	0.700	0.980	0.65
Glutamic acid	4.68	5.97	4.09
Glycine	0.560	0.650	0.47
Proline	1.50	1.44	1.31
Serine	0.662	0.930	0.51

\* an average number for all the wheats in the USDA report was used; \*\*European scale on dry matter

Table 6. Nutritional values for common wheat\*, Kamut® brand wheat (both available at [www.kamut.com](http://www.kamut.com)) and Graziella Ra wheat (present paper).



consensus trees (Figs. 6a and 6b) showed that molecular clustering disagreed with morphological clustering, in fact, contrary to AFLPs and SSRs, phylogenetic analyses of  $\alpha$ -gliadin gene sequences showed *Graziella Ra* and *Kamut* in separate clusters. This finding not only confirms that the two wheats are related but also supports the hypothesis that, although being similar – at least in the little part of the genome investigated by molecular markers employed in this study – *Graziella Ra* and *Kamut* may be considered distinct accessions.

### Nutritional quality

Given that all parameters linked to nutritional qualities are affected by the environment and that we compared *Graziella Ra* (analysed in triplicates) with *Kamut* (whose nutritional quality is reported in the *Kamut* web site, without any descriptions of how each parameter was assessed) a real comparison (including statistics) was not possible. Nevertheless, it is noticeable that all values of dietary components of *Graziella Ra* are in line with mean values reported for *Kamut* and other commercially available durum wheats (Table 6). Hence, our results corroborate the idea that *Graziella Ra* may be considered an accession distinct from *Kamut* endowed by appreciable levels of genetic diversity and medium-high nutritional qualities.

### ACKNOWLEDGEMENTS

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## Description of three new species of longhorn beetles (Coleoptera, Cerambycidae) from Turkey and Syria

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**ABSTRACT** The following new taxa are described and illustrated: *Chlorophorus grosseri* n. sp. from Southern and Eastern Turkey, close to *C. adeli* Holzschuh, 1974 from Western Iran; *Chlorophorus oezdikmeni* n. sp. from Turkey compared to *C. hungaricus* Seidlitz, 1891 and *Leiopus wrzecionkoi* n. sp. from North-Eastern Syria, compared to *L. syriacus* (Ganglbauer, 1884).

**KEY WORDS** Cerambycidae, longhorn beetles, new species, Turkey, Syria.

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### INTRODUCTION

Thanks to the courtesy of some colleagues we were able to study the Cerambycidae collected by them during their trips in Near Orient, including new taxa which have recently been published by ourselves (Rapuzzi & Sama, 2010; Rapuzzi et al., 2011). The aim of this article is to describe two new species belonging to the genus *Chlorophorus* Chevrolat, 1863 (Cerambycidae, Clytini) discovered in Turkey by our colleague Semra Turgut (entomologist at the Gazi University, Ankara) and by the Czech entomologist Walter Grosser respectively, as well as a new species of *Leiopus* Audinet-Serville, 1835 (Cerambycidae, Acanthocinini) collected by Antonin Wrzecionko.

#### *Chlorophorus grosseri* n. sp.

**MATERIAL EXAMINED.** Holotype female (Fig. 1): Turkey, Sirnak prov.: Mesindagi geç., 25 Km NW Sirnak, 1600 m, 37°67'N 42°31'E, 23.VI.2010, Walter Grosser legit; paratypes: 1 male (not available for detailed study): same data as the holotype; 1 female: Hakkari prov., 25 Km E Güzeldere, 37°32'N 43°49'E, 930 m,

22.VI.2010, Walter Grosser legit; 7 males and 8 females (immature adults just emerged ex larvae and pupae): same locality as the holotype, 15.V.2011, ex larvae and pupae in *Quercus* sp., P. Rapuzzi & G. Sama legit. Holotype in P. Rapuzzi collection; paratypes in W. Grosser, P. Rapuzzi, G. Sama and J. Vořísek collections.

**DESCRIPTION OF THE HOLOTYPE.** body length 9 mm. Integument reddish brown, the apical third of elytra, the hind legs and the ventral face of body black-brown. Front with a distinct median groove between the antennal tubercles. Pronotum strongly globose, as long as wide, discal surface with quite denser rasp-like punctures and sparsely clothed with fine greyish recumbent pubescence; this pubescence is chiefly condensed at sides as well as on front and, more widely, on basal margin. Scutellum densely clothed with white pubescence. Elytra moderately short and wide, apex truncate with a small tooth on the outer side; surface predominantly reddish-brown (black-brown on apical third only) with a pattern of distinctly contrasting stripes of white pubescence (see Fig. 1); discal surface very densely and finely punctate and clothed with short recumbent black pubescence.



Figure 1. *Chlorophorus grosseri* n. sp. holotype female.



Figure 2. *Chlorophorus grosseri* n. sp. paratype male.

Ventral side of body brownish-black, meso- and metepisterna, base of the metasternum and base of the first and second visible sternites densely clothed with contrasting white pubescence. Antennae reddish-brown, short, hardly extending to the middle of elytra, third to fifth joints sparsely clothed with erect hairs on latero-ventral surface. Front legs reddish brown, middle femora with claws blackish, hind legs black.

VARIABILITY. The male (Fig. 2) differs from the female by its more elongate pronotum, similar to *C. adellii* Holzschuh, 1974; the female paratype does not show difference except the length, 10 mm.

ETIMOLOGY. the new species is named in honour of our friend Walter Grosser from Czech Republic, who collected the first specimens.

DISTRIBUTION AND ECOLOGY. At present, the new species is known from Southern and Eastern Turkey. Larval bionomics similar to *C. adellii*, *C. ringenbachii* Sama, 2004 from Libya and *C. favieri* Fairmaire, 1873 from Morocco; oviposition takes place on dead apical part of small living branches or stumps (2-5 cm in diameter) cut by people the previous year or girdled by other Cerambycidae.

COMPARATIVE NOTES. *C. grosseri* n. sp. is closely related to *C. adellii* Holzschuh, 1974 from Zagros Mountains (western Iran) (male and female paratypes examined). This latter can be easily distinguished as follows: pronotum, in both sexes, longer than wide, sub parallel-sided, elytral integument predominantly black, brown on basal third only, antennae somewhat more robust, with proximal segments in average more elongate and distal segments evidently shortened.



***Cholorophorus oezdikmeni* n. sp.**

MATERIAL EXAMINED. Holotype male (Fig. 3) and three paratypes males: Karaman Marash prov., Andirin, 15.VII.2003, S. Turgut legit. Holotype in P. Rapuzzi collection; paratypes in H. Özdikmen (Gazi University, Ankara) and G. Sama collections.

DESCRIPTION OF THE HOLOTYPE. Body length 10 mm, entirely black except two dark-red spots on the pronotal disc and the elytral pattern. Front subquadrate with an unpunctate median area with a thin median groove. Pronotum as long as wide, globose, densely clothed with irregular vermiculate punctures and long white erect hairs, entirely black except one small, indistinct reddish spot on each side of the middle of the disc. Scutellum rounded, bordered with dense white pubescence. Elytra sub parallel-sided, black-brown, clothed on basal third with numerous erect white hairs and a pattern of whitish pubescence similar to *C. hungaricus* Seidlitz, 1891. Antennae short, hardly exceeding the middle of elytra.

VARIABILITY. Female unknown. Paratypes males: length varies from 9 to 12 mm; the red pronotal spots varies in size and shape: they can be very reduced like in the holotype, fused in a discal "M" shaped drawing or extended as a thin oblique line on each side of the disc.

ETIMOLOGY. we are pleased to dedicate the new species to our friend and colleague Huseyin Özdikmen (Gazi University, Ankara), for the authorisation to study the material belonging to his collection and for various help during our research on Turkish Cerambycidae.

DISTRIBUTION AND ECOLOGY. *C. oezdikmeni* n. sp. was collected in South-Western Turkey. Larval biology is unknown.

COMPARATIVE NOTES. *C. oezdikmeni* n. sp. belongs to the *C. trifasciatus* (Fabricius, 1781) species group; because of its pronotum and elytral base clothed with long erect hairs it is similar to *Chlorophorus hungaricus* Seidlitz, 1891, from which it can be immediately distinguished by its almost entirely black pronotum.

***Leiopus wrzecionkoi* n. sp.**

MATERIAL EXAMINED. Holotypus male (Fig. 4): Syria, Slinfah, Jabal An Nusayriyah [written as "Jabal An Nusaynyah" on labels], 1,300-1,800 m, 18.IV.2010, ex larva from *Alnus* sp., A. Wrzecionko legit; paratypes: eighteen males, five females: same data as the holotype; sixteen males, two females: Syria, Jabal An Nusayryah, Slinfah, 1,300-1,800 m, 27.IV.2008, A. Wrzecionko legit; one male: Slinfah; "the ridge above the town", 25.V.2005, D. Šanc legit, "The imago was beaten from the dry oak twig attached to the living tree". Holotype in P. Rapuzzi collection, paratypes in P. Rapuzzi, G. Sama, D. Šanc, A. Wrzecionko and Z. Košťál collections.

DESCRIPTION OF THE HOLOTYPE. Body length 8 mm. Integument black, pronotum and elytra densely clothed with greyish recumbent pubescence, third to tenth antennal joints more or less widely reddish at base. Head black with front sparsely clothed with white hairs, vertex with a deep impression between the antennal insertions. Pronotum transverse with an acute short tooth directed backward on each side just behind the middle, discal surface marked with numerous spots of black pubescence. Elytra short, somewhat flattened chiefly toward the apex and the sides, attenuate apically; discal surface clothed with short cinereous pubescence not masking the ground punctation and marked with a distinctly contrasting pattern consisting of numerous black round spots (each one originating a very short oblique seta) irregularly distributed on the basal and the apical quarter and along the suture, a median large black band narrowly interrupted near the suture and a longitudinal band entirely covering the epipleurae and the lateral margin of elytra. Legs and tarsi black, sparsely clothed with whitish pubescence locally condensed forming a median ring on tibiae and tarsi. Antennae long, exceeding the elytral apices with six segments.

VARIABILITY. The specimens we could study show a range of length between 9 to 11 mm. Pronotal and elytral black spots and stripes are sometimes more extended or reduced like in other species of the genus.



Figure 3. *Chlorophorus oezdikmeni* n. sp. holotype male.

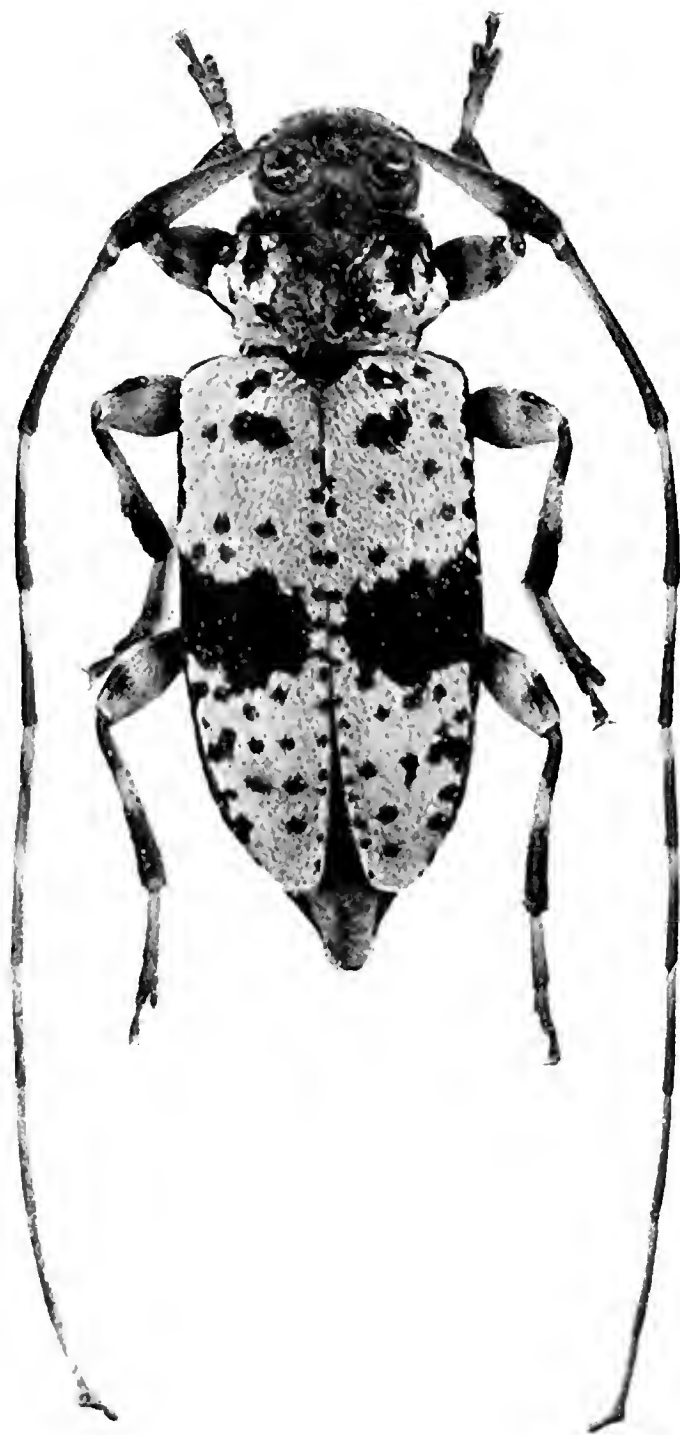


Figure 4. *Leiopus wrzecionkoi* n. sp. holotype male.

ETYMOLOGY. We are pleased to dedicate this new species to our friend Antonin Wrzecionko who discovered it.

DISTRIBUTION AND ECOLOGY. *L. wrzecionkoi* n. sp. was collected from North-Eastern Syria. Most specimens were collected in dead branches of *Alnus* sp. or by beating dried oak twigs.

COMPARATIVE NOTES. Despite its resemblance to *L. punctulatus* (Paykull, 1800) from Europe, due to its black body and its elytral pattern, *Leiopus wrzecionkoi* n. sp. belongs to the *L. syriacus* (Ganglbauer, 1884) species group. It is chiefly similar to *L. syriacus abieticola* Sama & Rapuzzi, 2010 from southern Turkey which can be distinguished from the new species by the integument constantly light-brown instead of piceous-black and the elytra distinctly convex.

## ACKNOWLEDGEMENTS

We wish to thank our colleagues and friends Antonin Wrzecionko (Horní Sucha, Czech Republic), Walter Grosser (Opava, Czech Republic), Huseyin Özdikmen and Semra Turgut (Gazi Üniversitesi, Fen-Edebiyat Fakültesi, Biyoloji Bölümü, Ankara) who kindly sent material of their collections for identification.

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# Does diet in lacertid lizards reflect prey availability? Evidence for selective predation in the Aeolian wall lizard, *Podarcis raffonei* (Mertens, 1952) (Reptilia, Lacertidae)

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**ABSTRACT** In this paper the invertebrate fauna occurring on Scoglio Faraglione, a tiny Aeolian island (Aeolian Archipelago, NE Sicily) inhabited by a population of the critically endangered lacertid lizard *Podarcis raffonei* (Mertens, 1952), was censused at different seasons and the resulting data were then compared with data obtained analysing prey composition and prey abundance in the diet of the lizards occurring on the same islet. The diet of *Podarcis raffonei* was mainly based on insects and other arthropods. The results indicate that diet composition is not directly influenced by prey availability and temporal prey abundance, and that there is strong evidence indicating selective predation. Lizards prey upon a number of arthropod categories fewer than that recorded in field. Some invertebrate taxa (e.g. Diptera and Gastropoda) are really less attractive for lizards and are rarely preyed or not preyed at all despite their spatial and/or temporal abundance. This suggests that *Podarcis raffonei* is able to operate a hierarchical choice within the range of prey items constituting its prey spectrum, probably through the ability to discriminate between prey chemicals or visually oriented predation.

**KEY WORDS** *Podarcis raffonei*; Lacertidae; predator selectivity; prey availability; feeding behavior; Aeolian Islands.

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## INTRODUCTION

Most lacertid lizards of the Mediterranean area are known to be active foragers and generalist predators (see e.g. *Podarcis siculus*: Kabisch & Engelmann, 1969; Pérez-Mellado & Corti, 1993). They prey on a wide variety of invertebrates, mainly on arthropods (e.g. Arachnidae, Insects larvae, Diptera, Coleoptera, Heteroptera, Hymenoptera, Orthoptera, Gastropoda) (see e.g. Capula et al., 1993; Rugiero, 1994; Corti & Lo Cascio, 2002; Bonacci et al., 2008; Corti et al., 2011), while occasionally small vertebrates and vegetal matter can be also eaten (Sorci, 1990; Sicilia et al., 2001; Capula & Aloise, in press). The feeding behavior of some lacertid lizards seems to be opportunistic, as indicated by the consumption of different preys in different habitats and/or geographic areas by the same species. However, few data are

available on predator selectivity and prey choice as well as prey availability in the field (see e.g. Heulin, 1986; Domínguez & Salvador, 1990; Maragou et al., 1996; Adamopoulou & Legakis, 2002; Pérez-Mellado et al., 2003; Bonacci et al., 2008). Hence special attention should be devoted to study selective predation and how diets of lacertid lizards relate to changes in the abundance of their prey, especially in micro-insular habitats, which are generally affected by extreme poorness of trophic resources and where lizards are usually assumed to be adapted to exploit the widest range of preys, alternatively adopting opportunistic or generalist feeding strategies (Pérez-Mellado & Corti, 1993; Carretero, 2004; Luiselli, 2008).

*Podarcis raffonei* (Mertens, 1952) is a lacertid lizard endemic to the Aeolian Archipelago (NE Sicily), where it occurs with four relict populations on three tiny islets

(Strombolicchio, Scoglio Faraglione, La Canna) and on a very small area of Vulcano Island (Lo Cascio, 2010; Capula & Lo Cascio, 2011). The conservation status of this species has recently received attention because it is likely threatened with extinction (Capula et al., 2002; Capula, 2006; Lo Cascio, 2010; Capula & Lo Cascio, 2011). As most of Mediterranean island lacertid lizards (see e.g. Pérez-Mellado & Corti, 1993; Van Damme, 1999), the diet of the Aeolian wall lizard is known to be based mainly on insects and other arthropods, but also includes variable percentages of vegetal matter (Luiselli et al., 2004; Lo Cascio, 2006; Capula & Lo Cascio, 2011). However, no data are available concerning prey choice and prey availability for the species.

The main aim of this study was to explore whether *P. raffonei* selects preys in accordance with their availability in the environment. To test this, the invertebrate fauna occurring on Scoglio Faraglione, which is an Aeolian tiny islet inhabited by *P. raffonei*, was censused at different seasons, and the resulting data were then compared with data obtained analysing prey composition and prey abundance in the diet of the lizards occurring on the same islet.

## MATERIALS AND METHODS

### *Study area*

Scoglio Faraglione (38°34'77" N – 14°48'08" E of Greenwich) is an uninhabited tiny islet of the Aeolian Archipelago. It lies in the Pollara Bay, 300 m off the western coast of Salina Island. The surface is 5,765 m<sup>2</sup> and the maximum altitude is 33 m a.s.l. The islet is composed by basaltic lavas, and was definitively isolated from the main island about 15,000-10,000 years ago, due to erosive processes, changes in eustatic sea level which occurred after the Last Glacial Maximum, and catastrophic eruption of the Pollara crater (13,000 yrs B.P.), which involved most part of the western slope of Salina Island and destroyed its original extension (Calanchi et al., 2007). Average annual rainfall (on the main island) is about 600 mm, with a peak in December and a minimum in July; average temperatures range from 13.3 °C (January) to 29.8 °C (August). The top of the Scoglio Faraglione islet is covered by dense shrub

vegetation, which is characterized by the occurrence of *Senecio cineraria* ssp. *bicolor*, *Dianthus rupicola* ssp. *aeolicus*, and *Lotus cytisoides*, while the rocky slopes of the basal belt harbour halo-chasmophytic plant communities dominated by *Limonium minutiflorum* and *Inula crithmoides*. Apart from the lizards, the only vertebrates that inhabit the islet are the Moorish gecko, *Tarentola mauritanica*, a small colony of Yellow-legged gull, *Larus michahellis*, and few pairs of other seabird species. As to the invertebrate fauna of Scoglio Faraglione, a non-exhaustive list is given by Lo Cascio & Navarra (2003).

### *Study lizards*

The population of *Podarcis raffonei* occurring on Scoglio Faraglione islet is characterized by medium-sized lizards with brownish dorsal coloration and ventral parts pearl-grey; it is referred to the ssp. *alvearioi* and is morphologically relatively differentiated from the populations of the same subspecies occurring on La Canna islet and Vulcano Island (Capula et al., 2009). Lizards are observed especially on the top of the islet, and are active mainly from March to November; however, occasional activity may be recorded also in sunny days during Winter. The activity pattern is unimodal in Spring and Autumn, and bimodal in Summer (Lo Cascio, 2006). The density of lizards ranges from 0.18 to 0.37 individuals/m<sup>2</sup>, and the estimated population size is about 300 individuals (Lo Cascio, 2006; Capula & Lo Cascio, 2011).

### *Sampling and taxonomic identification*

Field sampling was carried out during three visits in May, July, and October 2005. For the invertebrates, two sampling areas per session were selected on the top of the islet; each was 1 x 1 m sized. A better procedure would have required to seal completely the sample-area, using a biocenometer of 1 m<sup>3</sup> (see Pérez-Mellado et al., 2003), in order to collect all the animals occurring on soil, on vegetation and aerial parts inside the box. However, taking into account the fragility of the studied ecosystem, the peculiar vegetation pattern, and the morphology of the islet, a different methodological protocol was



adopted, following some of the proposals summarized by Disney (1986) and Ausden (1996). Into each sampling area invertebrates were collected i) by direct searching on substrate, under stones and on plants, using a pooter; ii) taking samples of soil and plant debris up to 10-15 cm depth, which were then examined and hand sorted in laboratory; iii) by sweep netting and beating on foliage; also, a plastic yellow Moericke trap (40 cm of diameter, filled by water and detergent to decrease the surface tension) was placed at the same level of the higher layer of vegetation for 5-6 hours, corresponding to the timeframe of lizards' activity. All the collected specimens were preserved in alcohol, except for Coleoptera, which were stored as dry material in the collection of one of the authors (PLC) and used for further studies. The taxonomic identification of the invertebrate fauna samples collected was performed comparing material preserved in the entomological collections of the Zoological Museum of Florence "La Specola". In the present analysis, the representatives of the invertebrate fauna were identified to OTUs (Operative Taxonomic Units: see Sneath & Sokal, 1973; Carretero, 2004), approximated to class/order level; the identification to OTUs at the family level was only performed for Coleoptera Melyridae and Hymenoptera Formicidae, because of the importance of these taxa in the diet of the local population of *P. raffonei* (Lo Cascio, 2006). The following abbreviations were used to indicate the OTUs in the text and figures: ACA, Acarina; ARA, Araneae; ART, unidentified Arthropoda; CHI, Chilopoda; CLB, Collembola; COL, Coleoptera; DPL, Diplopoda; DPT, Diptera; FOR, Hymenoptera Formicidae; GAS, Gastropoda; HET, Heteroptera; HOM, Homoptera; HYM, Hymenoptera; ISO, Crustacea Isopoda; LAR, insect larvae; LEP, Lepidoptera; MEL, Coleoptera Melyridae; NEM, Nematoda; NEU, Neuroptera; ODO, Odonata; PSE, Pseudoscorpiones.

Invertebrate fauna biomass was assessed using the following protocol: to each OTU was assigned a value (ranging from 0 to 10) which was estimated on the basis of its average size. For instance, the coleopterans occurring on the islet include about ten species, whose length ranges from 4 to 15 mm; the average size

calculated for that taxon was 5.5. The value assigned to each OTU was then multiplied with the total number of specimens collected in the field for each OTU. The diet of lizards (adult individuals only; snout-vent length (SVL)  $\geq 40$  mm) was studied on the basis of faecal pellets analysis. Faecal pellets were obtained from individuals captured in the field; after faecal pellets collecting, lizards were released in the site of capture (see Lo Cascio, 2006). Faecal contents were examined in the laboratory under stereoscope (10-40 X); item counting was based on the analysis of cephalic capsulae, wings, and legs, following the minimum numbers criterion by sample. The invertebrate remains were identified to OTUs at class/order/family level, as above mentioned.

### Statistical analysis

The diversity of prey item OTUs and invertebrate fauna OTUs collected in the field was calculated using Shannon Index (Shannon, 1948; see also Chao & Shen, 2003). Statistical analyses were performed using SPSS<sup>®</sup> version 11.5 for Windows PC package, with alpha set at 5% and all test being two tailed.

## RESULTS

The diet of lizards was composed mainly by arthropods, although plant matter was also recorded. A total number of 95 remains of arthropod preys were obtained from 34 faecal pellets of lizards at the study area. The composition and abundance of prey items and their temporal variations are summarized in Table 1. The identifiable preys (i.p.) were  $2.94 \pm 1.87$  per faecal pellet; the i.p. number differed significantly among seasons (May:  $4.18 \pm 2.08$ ; July:  $2.75 \pm 1.98$ ; October:  $1.92 \pm 0.90$ ;  $F_{2,28} = 5.22$ ,  $P = 0.01$ ). The prey spectrum also varied in a statistically significant way among seasons ( $\chi^2 = 47.59$ ,  $df = 26$ ,  $P = 0.006$ ), and the diet of lizards was more diversified in October ( $H_s = 2.146$ ) and May ( $H_s = 2.058$ ) than in July ( $H_s = 1.898$ ); however, in the latter comparison prey diversity was estimated analysing total amount of consumed preys only, without considering their seasonal variation. Overall,  $N = 696$  invertebrates

belonging to 21 different OTUs were collected into the sampling areas (see Fig. 1). Sixty seven percent of the OTUs collected in the sampling areas (14 out of 21) were found as prey items of lizards (see Table 1). Formicidae (FOR), Coleoptera (COL+MEL), Hymenoptera (HYM) and Diplopoda (DPL) accounted for the great part of the dietary spectrum. FOR, HYM, ART and HET were found in the diet of lizards from May to October, while DPL were found in July and October, and COL+MEL in May and July only. The other preyed OTUs (ARA, DPT, GAS, HOM, ISO, LAR, PSE) occurred with low frequency in the diet of lizards. The following OTUs were never found as prey items: ACA, CLB, CHI, LEP, NEM, NEU, ODO. Among the highly preyed taxa, Coleoptera and Heteroptera were represented in the diet with a percentage higher than that observed in the field (COL, diet: 12.6%, field: 7.8%; HET, diet: 4.2%, field: 1.8%). Hymenoptera were represented in the diet with a percentage (11.6%) close to that observed in the field (13.4%), and Formicidae occurred with relatively high frequency in the diet of lizards regardless of the season. Some taxa were represented in the diet with low or very low

frequency despite their spatial/temporal abundance in the field. This is the case of Diptera, which constitute the 4.2% of the diet although representing the 19.5% of the invertebrate biomass on the islet, and Gastropoda, which constitute the 1% of the diet only although being the 5.1% of the invertebrate biomass at the study area. Moreover, some invertebrates which are widespread and abundant in the field, such as e.g. Acarina and Collembola, were not present at all in the diet of lizards.

To test any relationship between the biomass of both prey items really hunted by lizards and potential prey items occurring in the field, the estimated biomass of the OTUs constituting the prey spectrum of lizards was compared with that of the OTUs sampled in the field. The comparison shows that the two groups are significantly different to each other ( $\chi^2 = 34.20$ ,  $df = 13$ ,  $P = 0.001$ ), thus suggesting little or no relationship. The estimation of the Shannon index gives similar values for both groups (hunted prey items:  $H_s = 2.265$ ; potential prey items:  $H_s = 2.269$ ), indicating a relatively high amount of diversity within each group.

Taxon	May	July	October
Araneae	10	-	6
Arthropoda (unidentified)	2	14	14
Coleoptera s.l.	19	5	-
Coleoptera Melyridae	28	5	-
Diptera	4	-	8
Diplopoda	-	23	22
Gastropoda	2	-	-
Heteroptera	2	10	6
Homoptera	2	5	-
Hymenoptera s.l.	6	24	6
Hymenoptera Formicidae	19	14	23
Insect larvae	2	-	5
Isopoda	4	-	5
Pseudoscorpiones	-	-	5

Table 1. Diet composition (in %) of *Podarcis raffonei* at Scoglio Faraglione Islet during 2005.

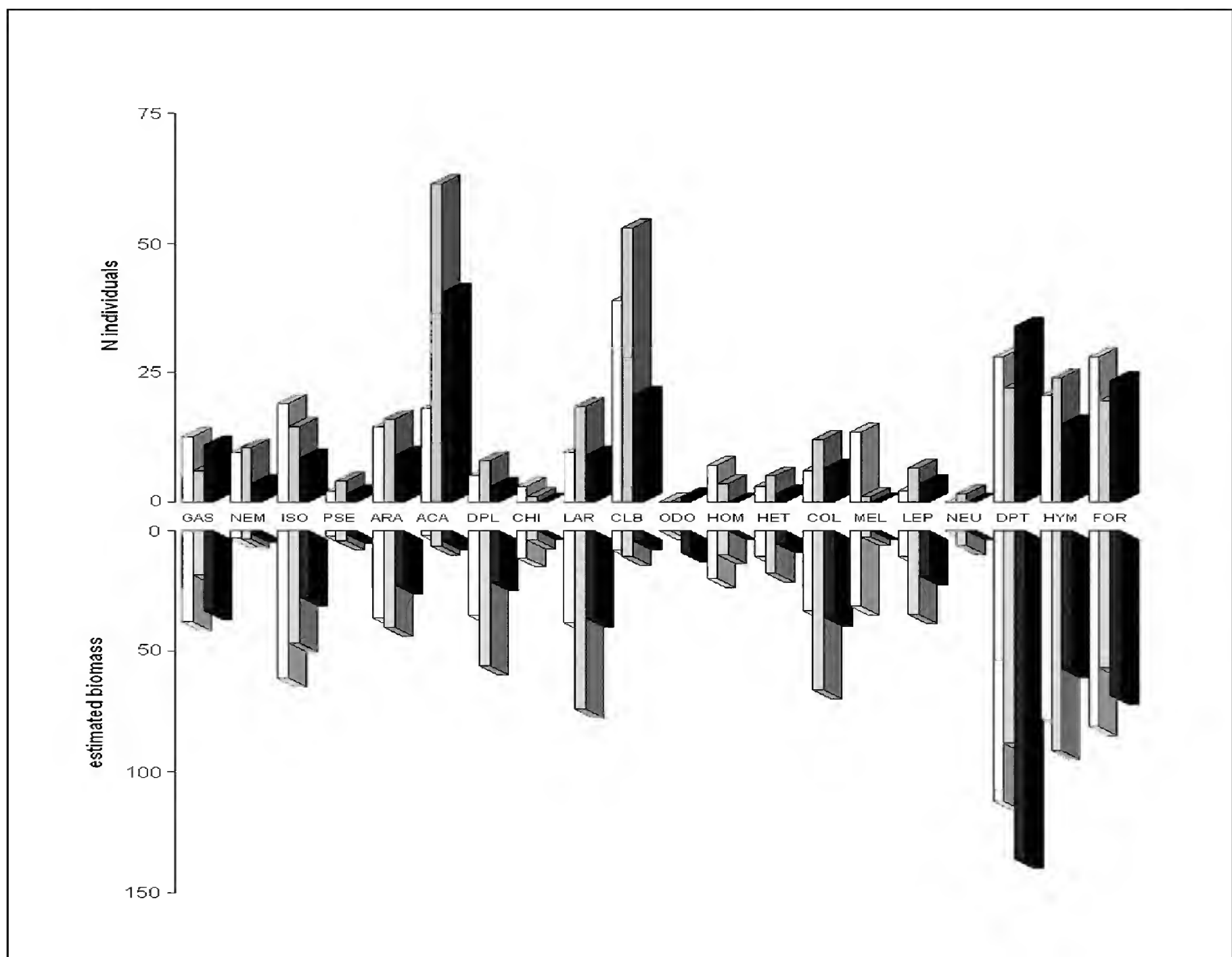


Figure 1. Frequency and estimated biomass of invertebrate fauna at the sampling areas during May (white histograms), July (grey) and October (black). Above: number of specimens collected in the field; below: estimated biomass of OTUs (see Material and methods for explanations).

## DISCUSSION

This study shows that in *P. raffonei* diet composition is not directly influenced by prey availability and temporal prey abundance, and that there is strong evidence indicating selective predation. These results suggest the occurrence of a food preference strategy similar to that observed in some lacertid lizard species (see e.g. Heulin, 1986; Domínguez & Salvador, 1990; Maragou et al., 1996; Adamopoulou & Legakis, 2002). Although caution should be exercised when inferring diet composition by faecal pellets analysis, as this methodology probably under-estimates the number of prey items and results depend on the number of samples collected and the subjectivity and taxonomic knowledge of the investigator, our

data indicate that some OTUs are really less attractive for lizards and are rarely preyed or not preyed at all despite their spatial and/or temporal abundance, probably because of prey chemicals or visual discrimination by lizards among possible prey items. This is the case of Diptera and Gastropoda, which were clearly neglected or rarely preyed by lizards, regardless of their abundance in the field, and Acarina and Collembola, which were never preyed by lizards, possibly because of the very small size (often less than 1 mm) of these arthropods, which cannot be considered as suitable preys for a medium-sized predator such as *Podarcis raffonei* (adult SVL of lizards ranging from ca. 40 to 80 mm).

Based on our results, it can be inferred that the Aeolian wall lizard is able to operate a

hierarchical choice within the range of prey items constituting its prey spectrum, probably through (i) the ability to discriminate between prey chemicals, or (ii) visually oriented predation. For instance, among the 14 OTUs usually preyed by the Aeolian wall lizard, Coleoptera, Heteroptera, Hymenoptera s.l. and Hymenoptera Formicidae can be clearly considered as preferred prey items by the species. In the case of Formicidae, it must be noted that myrmecophagy is a well-known feeding preference habit in island lizard populations (Pérez-Mellado & Corti, 1993; Adamopoulou et al., 1999; Carretero, 2004; Bombi et al., 2005; Lo Cascio & Pasta, 2006; Carretero et al., 2010). Diplopoda, which are known to produce a wide array of chemical defenses (see e.g. Blum & Porter Woodring, 1962; Duffey et al., 1977; Eisner et al., 1978; Kuwahara et al., 2002), apparently should not be considered as appetible preys by lizards. However, these arthropods can be found in the diet of Aeolian wall lizards from July to October with relatively high frequencies (see Table 1), and are completely missing as prey items in the periods of higher availability of most “appetible” preys, such as e.g. Coleoptera Melyridae, which not by chance are highly represented in the diet (and in the field) during Spring.

The analysis of the dietary spectrum of *Podarcis raffonei* clearly indicates that the species – differently from several *Podarcis* lizards occurring on western Mediterranean islands (Pérez-Mellado & Traverset, 1999; Van Damme, 1999) – consumes a low amount of plant matter (see also Luiselli et al., 2004; Lo Cascio, 2006) and can be considered as an opportunistic and mainly insectivorous predator. Although our results allow to hypothesize the occurrence of both visual and chemical discrimination of preys by the Aeolian wall lizard, at present we cannot say anything about the behavioral responses to the different kinds of prey and the chemicals involved in prey discrimination by *P. raffonei*. Further studies should thus be needed to investigate on the ability of the species to discriminate repellent chemicals and/or warning odours produced by several kinds of prey, and the senses that mediate this ability.

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## Observations on the genus *Athis* Hübner, [1819] and description of a new species from Peru (Lepidoptera, Castniidae)

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**ABSTRACT** One new species of the genus *Athis* Hübner, [1819] from Peru (*Athis pirrelloii* n. sp.) is described and illustrated. The male, the preimaginal stages and the host plant are still unknown. Some additional informations about the genus *Athis* Hübner, [1819] and the congeneric species/subspecies are given.

**KEY WORDS** Lepidoptera, Castniidae, *Athis*, new species, Peru

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### INTRODUCTION

The recent studies on Neotropical Castniidae have provided many significant contributions to our knowledge of their eco-ethology, systematics and biogeography. In particular, these in-depth studies have also contributed to extend our knowledge of the Australian genus *Synemon* Doubleday, 1846, with twenty new specific entities currently being described (González et al., 2010), while the data available on the distribution and natural history of the only Asian genus (*Tascinia* Westwood, 1877), made up of four species, remain scant.

The majority of the studies have concerned mainly the distribution of the Neotropical taxa, especially in Venezuela (González, 1998, 1999, 2003; González & Romero, 1997; González et al., 2006), Trinidad and Tobago (González & Cock, 2004), Colombia (González & Salazar, 2003), Mexico (Miller, 2000; González et al., 2008), Peru (Vinciguerra & Racheli, 2006; Vinciguerra, 2008a; 2008b, 2008c), Ecuador (Racheli & Vinciguerra, 2006; Vinciguerra, 2010) and Hispaniola (Vinciguerra, 2008a).

A further contribution has been the description of two interesting endemisms: *Insigniocastnia taisae* Miller, 2007 (Ecuador, Esmeraldas), and *Zegara polymorpha* Miller, 2008, currently

known only in Colombia (Otanche). The latter displays a marked polymorphism and is “involved” in complex mimetic chains with *Heliconius wallacei*, the Danaids of the *Lycorea* genus and the heterocera of the *Pericopis* and *Dysschema* genera (*D. unifasciata*, *bivittata*, *formossimia*, and *joiceyi*) (Miller, 2008).

Frequently in the Castniid, in fact, the imago is characterized by bright or aposematic (rarely cryptic) coloration and “mimics” the Lepidoptera of the Papilionidae, Danaidae, Ithomiidae, Hesperidae, Lycaenidae and Pericopidae families, relationships that would deserve further analyses.

However, the difficulty in locating the Castniid makes it hard to carry out systematic and faunistic studies on them: owing to the behaviours tied to the eco-ethology of the imago (brief flying activity, extreme localization and territoriality, adults only sporadically approaching the ground), the Castniid are in fact heterocera that are notoriously “under-represented” in the museum and private collections (Lamas, 1995; González, 1999; Vinciguerra & Racheli, 2006).

Commenting their capture, Strand wrote (see Seitz, 1913): “*Dans la plupart des cas la capture des Castnies comme papillon est égalem assez difficile; c’est sur les fleurs qu’on la prend le plus facilement. Sur des arbres en fleurs j’ai pris assez*”

*souvent de bons expl. C. pallasia et quelques decussata. Une fois dans le filet l'insecte se demène si énergiquement que c'est bien rare qu'on russi à rapporter un expl. immaculé*".

The *Athis* Hübner, [1819] genus (Figs. 1-10) which the species currently being described is ascribable to includes, according to Lamas (1995), approximately fourteen – fifteen taxa, making it the largest member of the Castniidae family, which includes a total of eighty known species divided into thirty genera (González et al., 2010). The distribution is Neotropical (Mexico, Bolivia, Brazil, Peru, Panama, Venezuela and Trinidad) with three significant endemisms present in the Caraibic area, including *Athis pinchoni* Pierre, 2003 (Martinica), and *Athis axaqua* Fernández-Yépez, 1992 (Margarita Island, Venezuela).

In the Island of Cuba, *Athis* Hübner, [1819], appears to be absent. The *Athis inca orizabensis* (Strand, 1913), specimens preserved at the Field Museum of Natural History (Chicago) as part of the Herman Strecker collection, and labelled as originating from Cuba, were actually introduced accidentally from Mexico with the introduction of vegetable species containing chrysalides (González et al., 2010).

The *Athis* imago has triangular-shaped forewings, with two (or three) hyaline ocelli located in the sub-apical area, the apex is pointed or rounded, while the hindwings are brightly-coloured, in contrast with the forewings, which are, usually, cryptic or dark brown (Figs. 4-10).

The adults appear to have selectively diurnal habits.

From a morphological point of view, the most similar genera are the *Insigniicastnia* Miller, 2007 and *Hista* Oiticica, 1955. The latter has been the subject of a recent systematic review (Moraes et al., 2010), and includes two taxa: *Hista fabricii* (Swainson, 1823), and *H. hegemon* (Kollar, 1839). The *Hista* species, in fact, were originally included by Houlbert in the *Athis* genus and subsequently appended to the *Hista* genus by Oiticica (1955), the founder of the genus, who had christened it *Hista* using the anagram of *Athis*, expressly to highlight the similarities between the two.

Little is known about the eco-ethology of *Athis* and the larval stages are virtually unknown, as are the host plants on which the worms evolve, albeit two recent studies have shed light on its

distribution and systematic: the first by González (2004) and concerning Venezuela, and the second on the *inca* "group" (Miller, 1972).

González et al. (2008) have also analyzed a probable hybrid between *Athis inca orizabensis* (Strand, 1913) and *Athis inca inca* (Walker, 1854), proof of the hybridization, occurring in nature, of the two sub-specific entities.

New research has been carried out on the distribution of *Athis fusciorubra* (Houlbert, 1917) (Fig. 9), found in the Island of Trinidad (González & Cock, 2004) and of *Athis palatinus staudingeri* (Vinciguerra & González, 2011 currently in press) discovered in Costa Rica and previously known to exist only in Panama. The taxonomic rank of the latter is unclear since Lamas (1995) considers it a sub-specific entity of *A. palatinus*, while Miller (1995), a valid species. The status of *Athis thysanete* (Dyar, 1912) (Fig. 8), endemic to Mexico and only seldomly captured, is equally uncertain.

Owing to some considerable morphological differences, this taxon is presumably not ascribable to *Athis* (González, personal communication).

#### *Athis pirrello* n. sp.

EXAMINED MATERIAL. Holotypus female (Figs. 1, 2): Peru, Huànuco, Cueva de las Pavas, 21.III.1998, 650 m, local collector legit, in the author's collection.

DESCRIPTION OF THE HOLOTYPUS. Head and thorax, in the dorsal part, are light brown in colour and light yellow in the ventral part. The antennae are dark brown. Abdomen: in the dorsal part, grey-brown in the first three urites, then yellow-ochre; in the ventral part extremely light yellow. Upper surface. Forewings: Length of the forewing: 52 mm, triangular-shaped wings, straight margin and rounded apex. The cost, in proximity of the apical area, is clearly characterized by a "depression" rendering the aforementioned area considerably elongated. Presence of two hyaline ocelli (one of which is larger than the other), whose boundaries are marked in black, and that are located in the sub-apical area. General coloration: light brown, slightly darker in proximity of the costal area (on the internal margin). Two ocelli (joined) are located: one in the discal area and the other in the costal area. Postdiscal band (wavy): scarcely visible, with four darker spots parallel to the edge. Upper surface. Hindwings: dark brown basal area;



1

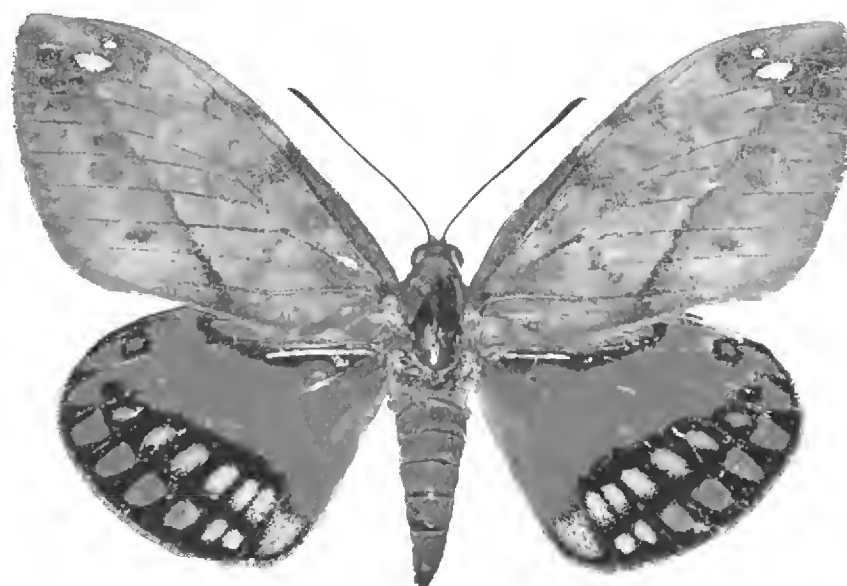


2

Figure 1. *Athis pirrelloi* holotypus female (recto): Peru, Huánuco, Cueva de las Pavas.  
Figure 2. *Athis pirrelloi* holotypus female (verso): Peru, Huánuco, Cueva de las Pavas.



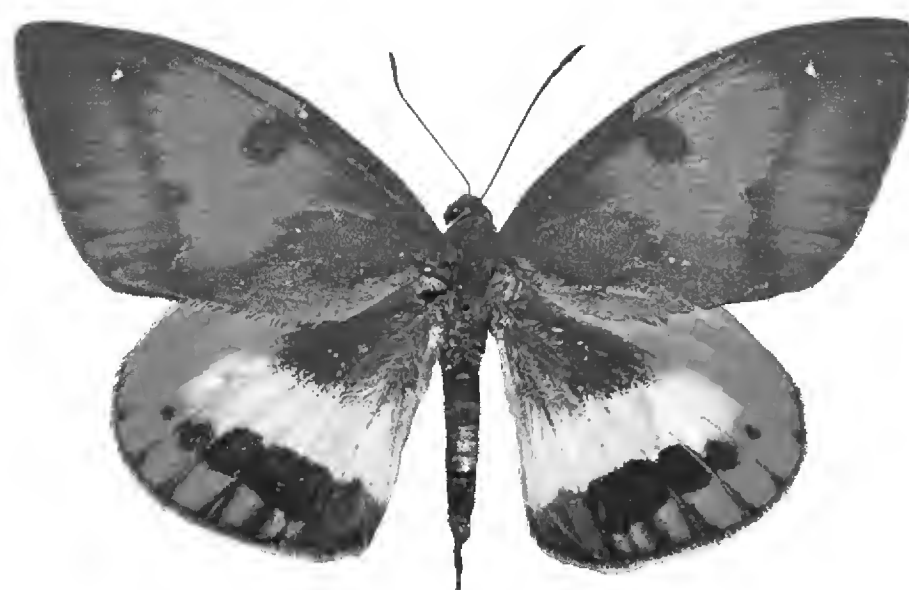
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Figure 3. *Athis rutila* female: Peru, Tingo Maria, Huánuco.

Figure 4. *Athis flavimaculata* male: Mexico, Jalisco, Tuxcacuesco.

Figure 5. *Athis palatinus staudingeri* male: Costa Rica, Corcovado.

Figure 6. *Athis palatinus ferruginosa* female: Peru, Tingo Maria, Huánuco.

extremely light yellow discal and postdiscal areas, marginal and costal areas orange-colored. Eight ocelli (the first two orange and the others dark brown) run parallel to the wing margin.

Lower surface. Forewings: yellow-ochre general coloration, darker compared to the upper surface, one ocellus is located in the discal area and another extends towards the costal area. On the lower surface, the postdiscal band is not visible. Hindwings: Uniform light yellow coloration. The eight ocelli, located on the upper surface, are barely discernible on the lower surface, except for the last two, which are located in proximity of the anal angle.

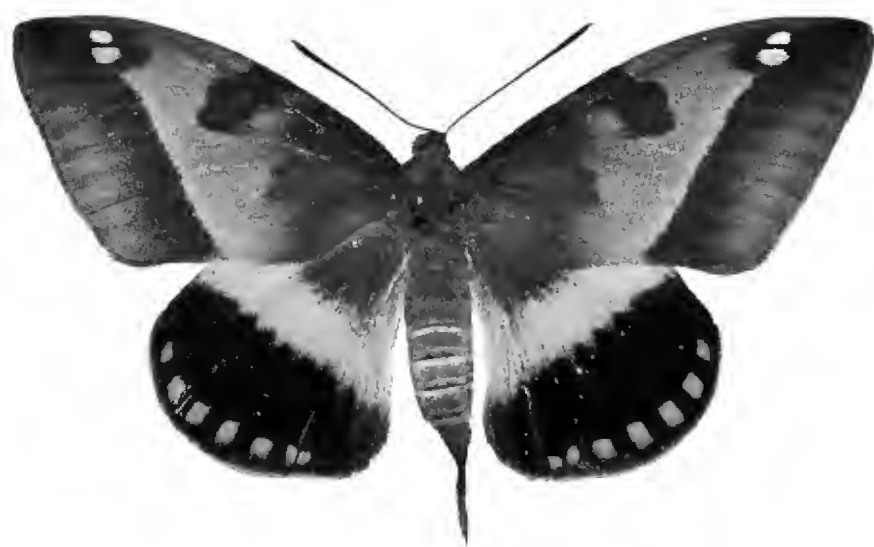
VARIABILITY. Male and other females are unknown, at present.

ETIMOLOGY. The species is dedicated to Roberto Pirrello (Trapani, December 24<sup>th</sup>, 1963), eminent surgeon, a Plastic and Reconstructive Surgery specialist, and a researcher and lecturer at the Faculty of Medicine and Surgery of the University of Palermo.

DISTRIBUTION AND ECOLOGY. Found only in its typical locality. The preimaginal stages and the host plant are still unknown.

COMPARATIVE NOTES. *Athis pirrello* n. sp. shares morphological and wing pattern similarities with the species of the *palatinus* "group" (Figs. 5-6). Clear analogies can be established with *Athis palatinus staudingeri* (Druce, 1896) (Panama and





7



8



9



10

Figure 7. *Athis superba* female: Peru, Tingo Maria, Huánuco.  
Figure 8. *Athis thysanete* male: Mexico, Puebla, Teuacan.  
Figure 9. *Athis fusciorubra* male: Peru, Satipo, Prov. Junin.  
Figure 10. *Athis therapon* male: Brazil, Santa Catarina, Joinville.

Costa Rica), which it differentiates itself from in terms of colouring and forewing shape.

The cost of *A. pirrello* n. sp., in proximity of the apical area, is clearly characterized by a “depression” considerably elongated, a peculiarity distinguishing it from all the other congeneric species and relating it to the female *Athis rutila* (Felder, 1874) (Fig. 3), which displays the same morphological characteristic.

In contrast with the other congeneric taxa, *Athis pirrello* has a considerably “elongated” forewing shape, a peculiarity it “shares” with *Athis therapon* (Kollar, 1839) (Fig. 10).

Kollar (1839) highlighted said peculiarity in the description of the *therapon* holotype, writing: “*Alis superioribus elongatis, supra flavescenti -*

*rufis [.....]*”, and also: “*Alae superiores haud consuetae plurimarum Castniarum formae, sed magis elongatae ...*”.

There are no other taxa with which to establish further comparisons, however, the hairs of the forewings and the study of the wing venation lead us to classify the species under the aforementioned genus.

## CONCLUSIONS

*Athis pirrello* constitutes an important naturalistic find worthy of further in-depth studies, which we intend to carry out when other specimens will be made available (extraction of DNA sequences, analysis of the genital

apparatus, study of the biogeographical distribution and of the variability of the species).

The holotype described and depicted below, and the specimens of the *Athis* genus shown, derive entirely from the author's collection.

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